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DEPARTMENT BULLETIN No. 1134

Washington, D. C. PROFESSIONAL PAPER April 26, 1923

SELF-FERTILIZATION AND CROSS-FERTILIZATION IN PIMA COTTON

By

THOMAS H. KEARNEY, Physiologist in Charge of Alkali and Drought Resistant Plant Investigations, Bureau of Plant Industry

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INTRODUCTION.

The three principal types of cotton grown in the United States—upland (*Gossypium hirsutum*), sea island (*G. barbadense*) and Egyptian[1]—hybridize freely among themselves when opportunity is afforded for cross-pollination. The first or conjugate generation of the hybrid between any two of these types is extremely fertile and vigorous, but in hybrids of upland with sea-island or with Egyptian cotton degenerate and more or less sterile forms occur in large numbers in the later generations (*29*).[2] On the other hand, so far as is known, the perjugate generations of crosses between varieties of the same type are little, if any, inferior in fertility to the parents. The high degree of compatibility between types so distinct as Egyptian and upland makes the frequency of natural cross-fertilization under given conditions a problem of much importance in breeding work with this plant and in maintaining supplies of pure seed of the agricultural varieties.

[1] The Egyptian type of cotton as it now exists appears to constitute a distinct botanical species, although it is supposed to have originated through hybridization (*27*, p. 289).

[2] Serial numbers (italic) in parentheses refer to "Literature cited" at the end of this bulletin.

Evidence is presented in this bulletin that although the cotton flower is admirably adapted to cross-pollination most of the ovules usually are self-fertilized. The percentage of vicinists, or natural hybrids, produced when two distinct varieties or types are grown side by side ordinarily is not large, although the occurrence of only a small initial percentage may, of course, seriously impair the purity of the stock. In the Egyptian type of cotton, particularly, self-fertilization has been found to predominate greatly over cross-fertilization. Investigations of the structure and later ontogeny of the flower, of the deposition of self pollen and of foreign pollen upon the stigmas, and of the competition of like and unlike pollens, here described, contribute to an explanation of the predominance of self-fertilization. Other aspects of the subject treated are the local and seasonal differences in the relative completeness of fertilization and the effect upon fertility of continued self-fertilization.

Most of the data and conclusions relate to the Pima variety of the Egyptian type of cotton, but comparison with upland cotton has been made in numerous instances. With very few exceptions the experiments were performed at Sacaton at the Pima Indian Agency in southern Arizona during the eight-year period from 1914 to 1921. Acknowledgment is made of the cordial cooperation of S. H. Hastings, formerly superintendent of the Cooperative Testing Garden at Sacaton, and of C. J. King, the present superintendent.

Many of the experiments from 1914 to 1919, inclusive, were performed by Walton G. Wells, during that period assistant cotton breeder in the Office of Alkali and Drought Resistant Plant Investigations, Bureau of Plant Industry. Walter F. Gilpin, assistant cotton breeder in the same office, who assisted in the work during 1917 and 1919, performed many of the experiments during the years 1920 and 1921. Others who have aided in the investigations are H. G. McKeever, Rolla B. Wade, Harvey Thackery, F. Ben Clark, Roy W. Nixon, George C. Powell, George J. Harrison, Robert D. Martin, C. J. King, W. W. Ballard, Max Willett, R. H. Manthey, R. H. Peebles, and C. A. Bewick.

Plates I, II, III, IV, and V are from photographs by W. F. Gilpin. Plates VI and VII are from photographs by Harold F. Loomis, of the Office of Crop Acclimatization and Adaptation Investigations, Bureau of Plant Industry.

VICINISM, OR NATURAL HYBRIDIZATION, IN COTTON.

In considering the evidence regarding the occurrence of vicinists, or natural hybrids, the published results of other investigators will be reviewed, and the data of experiments performed in Arizona will be presented.

DATA ON VICINISM IN LITERATURE.[3]

Webber, as the result of his experience in South Carolina and other Southeastern States, observes (*48*, p. 370):

> In several instances varieties have been grown in single rows with other varieties all around them of such a kind that crossing where it occurred could be easily detected in the progeny. Plants grown from seed matured under

[3] The accounts of experiments concerning vicinism in cotton rarely state whether or not the rows were thinned; and, if so, whether the removal of the extra plants was managed so as to avoid discrimination in favor of the more vigorous hybrid individuals.

such circumstances show but few crosses, indicating that the majority must have been self-fertilized. Judging from the observations thus far made, it would seem that ordinarily only from 5 to 10 per cent of the seeds are normally cross-fecundated.

Balls, on the basis of his investigations in Egypt, states (*6*, p. 27):

> The vast majority of individuals in any cotton crop yet studied are heterozygous in several characters, and the amount of crossing which takes place between cotton plants growing in a field so producing this heterozygous condition ranges from 5 to 25 per cent, by experimental evidence.

The same investigator, in a later publication (*7*, p. 222), remarks: "In 1905 we found that some 6 to 10 per cent of the ovules in a field of Egyptian cotton were cross-fertilized instead of being selfed;" and he points out that in general culture the apparent percentage of vicinists is usually larger than the actual percentage, owing to the stronger hybrid plants being retained when the fields are thinned. Certain progenies are mentioned (*8*, p. 119) in which the percentages of natural hybrids ranged from 25 to 35.

Allard (*2*) planted easily distinguishable varieties of upland cotton (Keenan, Okra Leaf, Red Leaf) in alternate rows in northern Georgia and found that progenies grown from at least 20 per cent of the bolls borne by plants of the Keenan variety contained one or more hybrids. Some of the bolls yielded only hybrids, indicating that the flowers from which these bolls developed had produced only abortive or self-sterile pollen.

Shoemaker (*43*), working in north-central Texas, found that when plants of the Triumph variety of upland cotton were scattered through a plat of an "okra-leaf" upland strain, so that each Triumph plant was entirely surrounded by plants of the other type, 47 per cent of the Triumph bolls, seed from which was planted the following year, yielded hybrids, although these in no case amounted to as much as 50 per cent of the entire progeny of the boll. The proportion of hybrids in the entire population grown from bolls collected on the Triumph plants was 10.9 per cent. No correlation could be observed between the position of the boll on the plant and the extent of the cross-fertilization observed, from which this investigator concluded that "the insects which did the crossing must have worked regularly through the season."

McLendon (*39*, pp. 162–167), in Georgia, grew Willett's Red Leaf and Hastings Big-Boll (a green-leafed variety of upland cotton) in alternate rows and planted seed from the Hastings plants. In a resulting population of 4,467, 87 (1.9 per cent) of the individuals proved to be vicinists.

Ricks and Brown (*1*, pp. 4, 15, 17), in Mississippi, found that when green-leafed varieties of upland cotton were grown in rows alternating with rows of Willett's Red Leaf, the percentages of natural hybrids produced by the resulting seed ranged from 4.9 to 11.1. From table 9 of the publication cited (p. 17) it may be deduced that of the bolls borne on plants of Lone Star and of Trice 36 and 44 per cent, respectively, gave progenies which contained one or more hybrids with Red Leaf.

In regard to the prevalence in India of natural cross-fertilization of cotton, Gammie (*19*, pp. 2, 3), from observations at Poona, concluded that it is a very rare occurrence. Evidence to the contrary

is given by Leake (*35*), by Kottur (*34*), and by Thadani (*46*). Kottur states that at Dharwar when two pure strains, one having a long leaf and the other a short leaf, were grown side by side, 6 per cent of vicinists occurred in the progeny of the short-leaf strain.

The distance to which pollen may be carried under natural conditions is a subject of much practical importance. Shoemaker (*43*) observed that where Triumph and Okra Leaf cottons were grown 2 rods apart,[4] a planting from the seed of the former yielded about 1 per cent of hybrids. This would indicate that a relatively slight distance affords a fair degree of protection against cross-pollination.

Balls (*8*, pp. 19, 123), in Egypt, found that whereas under ordinary field conditions the number of vicinists ranged from 5 to 10 per cent, in his breeding plat, where numerous different types of cotton were grown in close proximity, the percentage rose to as high as 50 or even 100. He observed that hybrids were occasionally produced with Willett's Red Leaf when the plants of the latter were 50 meters distant from the plants which produced the hybridized seed, with dozens of other cotton plants intervening.

Ricks and Brown (*1*), in Mississippi, found that seed gathered from plants of the Cleveland variety of upland cotton which were situated in the middle of a 4-row plat of this variety, the plat being separated by 10 rows of corn from a row of Willett's Red Leaf cotton, produced 0.8 per cent of Cleveland × Red Leaf hybrids, as compared with 4.9 per cent where the two varieties were grown in adjacent rows and 18.5 per cent where they were grown in alternate hills.

EXPERIMENTS IN ARIZONA.

VICINISM BETWEEN VERY DISTINCT TYPES.

A plat of cotton of the Egyptian type was grown by the writer at Yuma, Ariz., in 1907 in close proximity to a plat of upland cotton. Seed from the open-pollinated flowers of the Egyptian plants was planted in 1908, and of the resulting population of approximately 3,000 individuals 8.2 per cent were hybrid.

Under the direction of O. F. Cook, Egyptian and Kekchi (upland) cottons were planted in alternate rows by Argyle McLachlan near Yuma in 1909. The population grown in 1910 from the seed produced by the Kekchi plants contained 5 per cent of hybrids.[5]

Open-pollinated bolls were collected at Sacaton in 1919 from three adjacent rows of cotton, there having been a row of Pima (Egyptian), bordered on one side by a row of the Lone Star (upland) variety and on the other by a row of the Holdon (upland) variety. The seed obtained from each row was planted in 1920, and the percentages of first-generation hybrids were determined, as given in Table 1.

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[4] Although the point is not mentioned in the work cited, Dr. Shoemaker has informed the writer that to the best of his recollection there were several rows of Triumph cotton between the plants of that variety from which seed was gathered and the row of Okra Leaf cotton.

[5] Argyle McLachlan in letter to O. F. Cook, July 9, 1910.

TABLE 1.—*Hybrids in populations from open-pollinated seed produced by adjacent rows of Egyptian and upland varieties of cotton at Sacaton, Ariz., in 1920.*

Variety from which seed was obtained.	Plants.	F_1 hybrids.	
		Number.	Per cent.
Pima (Egyptian)	585	17	2.9±0.5
Lone Star (upland)	448	23	5.1± .7
Holdon (upland)	437	49	11.2±1.0

That these percentages of hybrids correspond closely to the actual percentages of ovules which were cross-fertilized by pollen of the other type is indicated by the following facts: The seeds were planted four to the hill and no thinning was done. Comparison of the percentages of hybrids in the hills containing one, two, three, and four plants, respectively, showed that while each successive increase in the number of plants was accompanied by a decrease in the percentage of hybrids, the differences were not significant, even as between hills containing one plant and hills containing four plants. Hence, it may be concluded that little, if any, natural selection in favor of the hybrid plants had taken place during germination and the seedling stage of growth.[6]

The difference in the percentages of hybrids between the progenies of the Pima and of the Lone Star plants is apparently not significant, but the percentage of hybrids in the progeny of Holdon is nearly four times as great as in the progeny of Pima, and the difference is $7\frac{1}{2}$ times its probable error. So far as this evidence goes, it would seem that when Egyptian and upland cottons are grown in close proximity, the former yields a smaller percentage of vicinists than the latter. It should be noted, however, that during the latter half of the summer the upland plants showed a much greater decline in the rate of flowering than did the Pima plants, and this would favor the production of a higher percentage of upland × Pima than of Pima × upland hybrids.

The extent of vicinism occurring when upland plants are located in the midst of a field of Egyptian cotton is indicated by the results of an experiment performed at Sacaton in 1920 and 1921. Fifty plants of Acala (upland) cotton were grown in 1920 in the middle section of the central row of a 7-row plat of Pima cotton. Adjacent to this plat, on both sides, were several other plats which contained only Pima cotton. The rows contained about 400 plants each, so that the upland cotton was completely surrounded by the Egyptian. The arrangement of the planting is shown in Figure 1.

[6] Data given elsewhere in this bulletin indicate that seeds produced by Pima flowers which had been cross-pollinated with upland pollen germinate somewhat better than Pima × Pima seeds, although the difference in the germination did not exceed 4.1±1 per cent and was therefore too small to affect materially the percentage of hybrids yielded by seed from naturally pollinated flowers on the Pima plants. To illustrate: If the proportion of germination of the seeds resulting from cross-fertilization of Pima ovules with upland pollen was only 4 per cent higher than that of seeds from Pima ovules fertilized with Pima pollen and if the population grown from seed produced by naturally pollinated Pima flowers contained 10 per cent of hybrids, the actual percentage of hybridized (Pima × upland) ovules would have been 9.6 per cent.

Bolls were gathered in the fall from all 50 of the Acala (upland) plants and from each of the 50 plants, opposite to these, in Pima rows 1, 2, 3, 4, 6, 8, 10, 15, and 20, on the west and rows 1′, 2′, 3′, 4′, 6′, 8′, 10′, 15′, and 20′ on the east of the upland. Each lot of seed was thoroughly mixed, and a representative sample was planted in

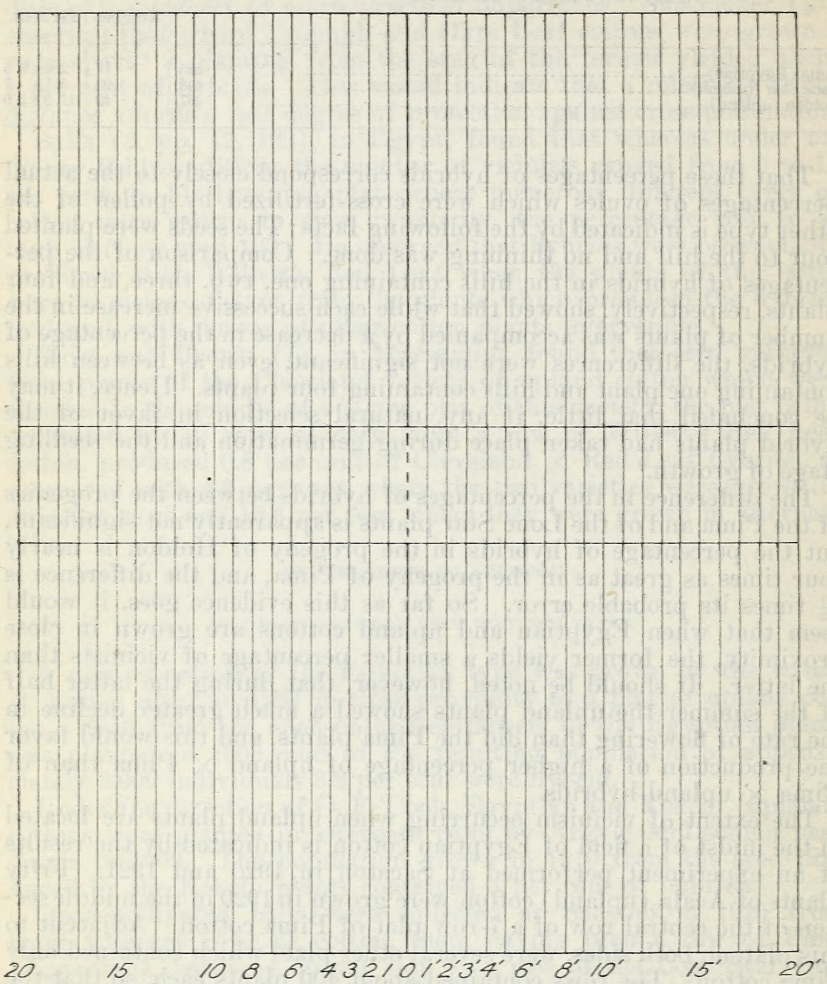


FIG. 1.—Planting plan of an experiment to determine the extent of vicinism resulting when plants of the Acala variety of upland cotton were located in the midst of a field of the Pima variety of Egyptian cotton. The middle section of the central row (row 0), indicated by the dotted portion of the line, contained the upland plants, the remainder of this row and the rest of the field having been planted to Pima. Horizontal lines inclose the portions of the Pima rows from which seed was harvested for determination of the percentage of vicinists.

1921 in order to determine the percentages of vicinists. No thinning was done, all seeds which germinated having been allowed to develop. The results are stated in Table 2, in which the populations grown from each pair of Pima rows having the same cardinal number and its prime, both east and west of the Acala section, are combined as one array.

TABLE 2.—*Vicinists yielded in 1921 by seed from plants of Acala cotton located in the midst of a field of Pima cotton and by seed from those portions of successive Pima rows which were opposite to and on both sides of the section of Acala cotton, at Sacaton, Ariz., in 1920.*

Variety from which seed was obtained.	Plants.	F_1 hybrids.		Variety from which seed was obtained.	Plants.	F_1 hybrids.	
		Number.	Per cent.			Number.	Per cent.
Acala (upland),[1] row 0	671 (714)	93 (136)	13.9±0.9 (19.1)	Pima (Egyptian)—Con.			
Pima (Egyptian):				Rows 6 and 6′	579	1	0.2
Rows 1 and 1′	635	9	1.4± .3	Rows 8 and 8′	456	0	0
Rows 2 and 2′	615	2	.3	Rows 10 and 10′	[2] 600	0	0
Rows 3 and 3′	685	4	.6	Rows 15 and 15′	[2] 600	0	0
Rows 4 and 4′	609	0	.0	Rows 20 and 20′	[2] 600	0	0

[1] Through an oversight, the seed from the Acala plants was planted in a plat in which upland cotton had been grown in 1920. There were numerous volunteer plants, many of them first-generation upland × Egyptian hybrids which could not be distinguished from the hybrids belonging to this experiment unless they occurred outside the rows and hills of the 1921 planting. Consequently, it was deemed best to count as vicinists belonging to this experiment only F_1 plants which grew in hills with plants of Acala, excluding such F_1 plants as occurred singly in a hill, even though their alignment and spacing distance conformed to that of the 1921 experiment. The figures obtained by including such plants are, however, given in parentheses. It is probable that the first percentage given in the table is lower and the second higher than the true percentage of vicinists yielded in this experiment.

[2] Estimated.

The percentage of vicinists yielded by the section of Acala plants was at least ten times greater than that yielded by the Pima plants which grew on either side of them (rows 1 and 1′). A considerably higher percentage in the former case would be expected (1) because the Acala was surrounded on all sides by Pima and (2) because during the latter part of the season the Pima plants were flowering more profusely than the Acala. But these factors alone do not seem adequate to explain the much greater proportion of vicinism in the case of the upland. It will be noted that no vicinism was detected in the Pima cotton situated farther away from the Acala than rows 6 and 6′.

A 7-row plat of Pima cotton was grown at Sacaton in 1920 adjacent to a 7-row plat of Durango (upland). Each row of each variety was harvested separately. Each resulting lot of seed was thoroughly mixed, and a portion of each lot was planted in 1921 in order to determine the percentages of vicinists. No thinning was done, all plants which germinated having been allowed to develop. The results are stated in Table 3.

TABLE 3.—*Vicinists yielded in 1921 by plantings of seed from successive rows in adjacent plats of Pima and Durango cottons grown at Sacaton, Ariz., in 1920.*

[No. 1 designates the adjacent row of each of the two varieties and No. 7 the row of each variety which was most remote.]

Row.	Plantings of seed from Pima (Egyptian) rows.			Plantings of seed from Durango (upland) rows.		
	Plants.	F_1 hybrids.		Plants.	F_1 hybrids.	
		Number.	Per cent.		Number.	Per cent.
No. 1	270	9	3.3±0.7	255	6	2.4±0.6
No. 2	386	8	2.1± .4	313	10	3.2± .7
No. 3	346	10	2.9± .6	288	2	.7± .3
No. 4	252	4	1.6± .5	313	2	.6± .2
No. 5	365	2	.5± .2	233	7	3.0± .8
No. 6	355	3	.8± .3	286	2	.7± .3
No. 7	361	5	1.4± .4	288	4	1.4± .5
Total	2,335	41	1.8± .2	1,976	33	1.7± .2

The results of this experiment are exceptional, practically the same total percentage of vicinists having been yielded by seed from the Egyptian and from the upland plants, and the percentage yielded by seed from the row of each variety which was most distant from the plat of the other type (row 7) having been not significantly lower than that yielded by the row that was nearest (row 1).

Seed was gathered in 1920 from each of a number of rows in a field of Pima (Egyptian) cotton at Buckeye, Ariz., which was separated from a field of upland cotton by a rather wide road bordered by a row of trees. Each lot of seed was thoroughly mixed, and a portion of that from each row was planted in 1921 in order to determine the percentages of vicinists. No thinning was done, all seeds which germinated having been allowed to develop. The results are stated in Table 4.

TABLE 4.—*Vicinists yielded in 1921 by plantings of seed collected in 1920 from several rows of Pima cotton situated in close proximity to a field of upland cotton at Buckeye, Ariz.*

[No. 1 designates the outside row, nearest the upland field.]

Row.	Plants.	F_1 hybrids.		Row.	Plants.	F_1 hybrids.	
		Number.	Per cent.			Number.	Per cent.
No. 1	284	6	2.1	No. 20	143	1	0.7
No. 2	314	2	.6	No. 30	138	1	.7
No. 3	305	1	.3	No. 40	136	3	2.2
No. 5	287	4	1.4	No. 50	285	5	1.8
No. 10	291	0	0				
No. 15	189	0	0	Total	2,372	23	1.0

It is interesting to note that the most distant row (No. 50) yielded a percentage of vicinists not materially lower than that yielded by row No. 1, which was nearest the field of upland cotton. It seems, however, that pollinating insects which had left one field and crossed a wide road bordered by trees would be as likely to alight at a considerable distance within the second field as at the edge of it.

VICINISM BETWEEN VARIETIES OF THE SAME TYPE.

The cases of vicinism in Arizona thus far discussed have been between widely different types of cotton. It will be interesting to consider a case involving two related varieties belonging to the same general type but sufficiently uniform and sufficiently distinct to make the recognition of accidental hybrids between them fairly certain. A row of Pima cotton was grown side by side with a row of Gila cotton, both varieties belonging to the Egyptian type,[7] at Sacaton, Ariz., in 1916. In this case there was no appreciable difference in the height of the plants and the duration of the flowering period. Seed from the open-pollinated Pima flowers was planted in 1917. The hills were thinned to one plant, the thinning having been done in such manner as to avoid selection. The total number of plants after thinning was 302, of which 5 were certainly and 2 more were doubtful first-generation Pima × Gila hybrids. The

[7] These varieties are described by Kearney (*27*), and the characters of hybrids between them are discussed by Kearney and Wells (*30*).

indicated maximum proportion of hybrids was therefore 2.3 per cent. Taken in connection with the low percentages of hybrids produced by seed from open-pollinated flowers of Pima cotton grown adjacent to upland cottons (Tables 1, 2, and 3) these data indicate a strong tendency to self-fertilization in the Pima variety.

VICINISM NOT A COMPLETE MEASURE OF CROSS-FERTILIZATION.

The percentage of recognizable vicinists does not afford an adequate expression of the relative frequency of cross-fertilization as compared with self-fertilization, for the plants produced by ovules which have been fertilized with pollen from other plants of the same variety are usually not distinguishable from the plants resulting from self-fertilization. In order to determine the actual percentage of ovules which have been cross-fertilized, a single individual of one variety should be isolated among plants of another and readily distinguishable variety, allowing only one flower to open daily on the isolated mother plant. In such case only recognizable hybrids would be produced by all seeds from ovules not fertilized with pollen of the same flower.

The conditions outlined in the preceding paragraph were met in an experiment begun at Sacaton, Ariz., in 1920. In the central row of a 7-row plat of Acala (upland) cotton 8 plants of Pima (Egyptian) were so located that from 5 to 10 Acala plants intervened between each 2 Pima plants. Eight plants of Acala cotton were similarly located in a plat of Pima. Only one flower was allowed to open daily on each of the isolated plants, any additional flower buds due to open on the same day having been removed before the corolla expanded. It is believed that under these conditions all or very nearly all of the ovules were either strictly self-fertilized or were cross-fertilized by pollen of the other type. Consequently, the total cross-fertilization which took place should be indicated by the percentages of first-generation hybrids in the progenies of these plants.

The seed produced by each of the isolated individuals was planted in 1921. No thinning was done, all seeds which germinated having been allowed to develop. The percentages of hybrids were determined after the plants had developed sufficiently to make identification certain. The results are stated in Table 5.

TABLE 5.—*First-generation hybrids in the progenies of Pima and of Acala plants which had been grown isolated in a plat of the other variety and on which only one flower had been allowed to open daily, at Sacaton, Ariz., in 1920.*

Pima (Egyptian) plants.				Acala (upland) plants.			
Progeny.	Number.	F_1 hybrids.		Progeny.	Number.	F_1 hybrids.	
		Number.	Per cent.			Number.	Per cent.
No. 1	263	4	1.5±0.5	No. 1	219	64	29.2±2.1
No. 2	340	26	7.6±1.0	No. 2	283	98	34.6±1.9
No. 3	211	33	15.6±1.7	No. 3	197	66	33.5±2.3
No. 4	259	30	11.6±1.3	No. 4	225	48	21.3±1.8
No. 5	157	47	29.9±2.5	No. 5	285	64	22.4±1.7
No. 6	234	28	12.0±1.4	No. 6	223	75	33.6±2.1
No. 7	212	11	5.2±1.0	No. 7	260	73	28.1±1.9
No. 8	242	52	21.5±1.8	No. 8	123	18	14.6±2.1
Total	1,918	231	12.0± .5	Total	1,815	506	27.9± .7

There was much more variation in the percentage of hybrids among the progenies of the Pima plants than among the Acala progenies, one of the Pima progenies having yielded a somewhat higher percentage than most of the Acala progenies. If the eight progenies of each variety are taken as a single population, however, it is seen that the percentage of cross-fertilized ovules was more than twice as great in the upland variety as in the Egyptian variety. The results of this experiment indicate that on the average 88 per cent of the ovules in Pima (Egyptian) cotton and 72 per cent in Acala (upland) cotton were autogamically fertilized.

These types of cotton differ less in height of plant and rate of flowering in early summer than later in the season, when the Pima plants become much taller than the Acala and produce relatively a greater number of flowers. In order to determine whether these differences are reflected in different degrees of cross-fertilization of the early and late flowers, dated tags were attached to all flowers which opened on the isolated plants. The progeny of each individual was planted in three sections, representing as many periods during which the flowers had opened—July 1 to 21, July 22 to August 11, and August 12 to September 3. The number and percentage of F_1 hybrids from seed produced by flowers which opened during each period were determined for each variety, these data being presented in Table 6.

TABLE 6.—*First-generation hybrids yielded in 1921 by seed representing different flowering periods which was produced by the isolated plants of Pima and of Acala cotton, at Sacaton, Ariz., in 1920.*

Period.	Progenies of 8 Pima (Egyptian) plants.			Progenies of 8 Acala (upland) plants.		
	Number.	F_1 hybrids.		Number.	F_1 hybrids.	
		Number.	Per cent.		Number.	Per cent.
July 1 to 21	311	63	20.2±1.5	646	166	25.7±1.2
July 22 to August 11	793	136	17.2± .9	462	120	26.0±1.4
August 12 to September 3	815	32	3.9± .5	706	220	31.2±1.2

The difference between the two varieties in the percentage of hybrids from seed produced by flowers of the first period probably was not significant, but flowers of the second and third periods yielded significantly greater percentages of hybrids in the case of Acala than in the case of Pima plants. The very marked decline during the last period (August 12 to September 3) in the relative cross-fertilization of the flowers borne by the isolated Pima plants is probably to be attributed to a diminished flower production of the Acala plants which surrounded them. The isolated Acala plants, on the other hand, showed a slight increase in the percentage of cross-fertilization during the same period, indicating that no corresponding reduction had taken place in the rate of flowering of the Pima plants by which they were surrounded.

The fact that the Pima and the Acala flowers which opened during the period from July 1 to 21, when both types of cotton were in full

blossom, yielded approximately equal percentages of hybrids points to the conclusion that the higher percentages of vicinists usually obtained from seeds produced by upland plants than from seeds produced by Egyptian plants when the two types are grown side by side is due partly to the earlier slowing down of the rate of flowering in the case of upland cotton. Evidence presented in another part of this bulletin indicates, however, that there may be an intrinsic difference in the liability to cross-fertilization of the two types.

CONCLUSIONS REGARDING THE PREPONDERANCE OF SELF-FERTILIZATION.

There is much variation in the percentages of vicinists, or natural hybrids, formed when two distinct types of cotton are grown in proximity, as is shown by the results obtained by other investigators and by the writer. This is to be expected in view of the many variable factors involved, such as local differences in the number and kind of pollinating insects and differences in the habit of growth and period of flowering of the varieties. The proportion of vicinists rarely exceeds 20 per cent, however, and is usually much smaller. The available information in regard to vicinism therefore points strongly to the conclusion that in cotton self-fertilization greatly predominates over cross-fertilization. It should not be inferred, however, that because most of the ovules normally are self-fertilized, such cross-fertilization as occurs is negligible in its effect upon the uniformity of a variety.

As a rule, the percentage of vicinists decreases rapidly as the distance between the seed-bearing and the pollen-bearing parents increases, but the data at hand do not permit a conclusion to be drawn as to the degree of isolation necessary to eliminate the danger of cross-pollination. This is doubtless affected by the nature of the varieties grown, by local and seasonal variations in the insect population and in the flowering of other plants, and by topography, weather, and other factors.

The percentage of recognizable vicinists produced under natural conditions does not measure the proportion of cross-fertilization occurring, for the reason that many of the ovules are cross-fertilized by pollen from other plants of the same variety. An experiment was performed at Sacaton, Ariz., in which this source of error was eliminated by growing scattered plants of one type in a field of another type and allowing only one flower to open daily on each of the isolated plants, seed from which was planted the following season. Plants thus treated yielded 12 per cent of hybrids in the case of Pima (Egyptian) and 28 per cent in the case of Acala (upland). It is believed that these percentages correspond very closely to the percentages of cross-fertilized ovules.

The results of this experiment indicated that in Pima 88 per cent and in Acala 72 per cent of the ovules were self-fertilized. Other evidence has been obtained at Sacaton that upland × Egyptian are more numerous than Egyptian × upland vicinists. That this is due partly to an earlier decline in the flowering rate of upland as compared with Egyptian cotton is suggested by the fact that seeds produced by flowers of Pima and of Acala cotton which opened during a period when both types were blossoming freely yielded approximately the same percentage of vicinists.

STRUCTURE OF THE FLOWER IN RELATION TO POLLINATION.

The large and showy cotton flower with its reproductive organs so placed as to be readily accessible to all kinds of insects (Pls. I and II) would seem to be admirably adapted to cross pollination, especially as the abundant secretion of nectar attracts large numbers of pollen-carrying insects. The transfer of pollen is favored by the fact that even during the height of the blossoming period the number of flowers opening daily on the individual plant rarely exceeds three and is usually only one.[8] Yet the evidence presented in the preceding pages indicates a strong preponderance of self-fertilization. In seeking an explanation of this apparent anomaly the structure and the later ontogeny of the flower will be considered.

The description which follows is based upon the Pima variety, but applies in all essential particulars to other varieties of the Egyptian type. The points of structure relative to pollination in which the flower of upland cotton (*Gossypium hirsutum*) differs from that of Egyptian cotton will be mentioned for comparison.

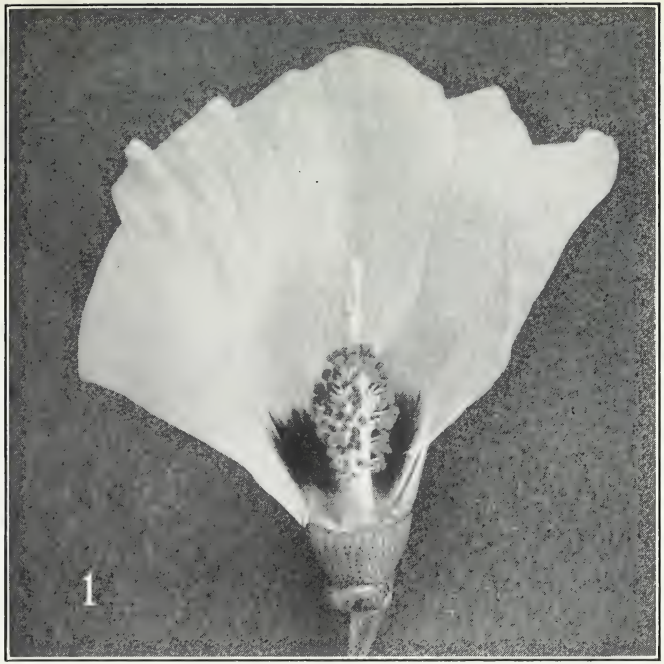
POSITIONAL RELATIONS OF THE REPRODUCTIVE ORGANS.

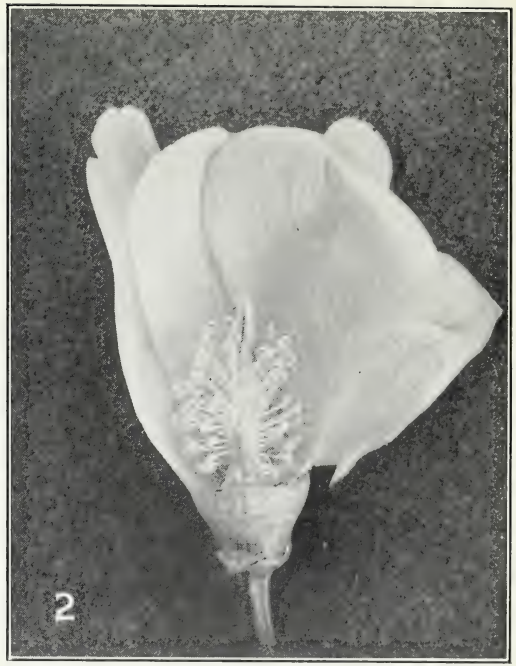
Egyptian cotton, like other members of the genus Gossypium, has the ovary and style inclosed in a sheath or tube formed by the coalescent bases of the filaments of the stamens, and the pistil projects above the summit of this so-called staminal column (Pl. I, Fig. 1; Pl. II, Fig. 1). There is no sharp differentiation between stigmas and style, the latter beginning to increase in diameter and to become pubescent below the summit of the staminal column, but under normal conditions pollen is deposited in quantity only on the exserted portion of the pistil, and for convenience the term "stigmas" will be used in referring to this portion only. The erect and usually somewhat spirally twisted stigmas (Pl. I, Fig. 1) are coherent except very near the apex and are slightly enlarged upward. The stigmatic surface is not viscid but is densely pubescent, and this together with the spiny surface of the pollen grains secures their adhesion to the stigmas. Unlike the condition in many of the Malvaceæ, the stigmas do not become spreading or reflexed after the flower opens but remain erect. There is no evidence that the flower is protandrous, as is the case in most of the Malvaceæ.[9] The stigmas from the apex to a little below the point where they emerge from the staminal column are homogeneous in texture and pubescence, and pollen grains adhere to and doubtless germinate upon all parts of their surface.

Reference to Plate I, Figure 1, and to Plate II, Figure 1, shows that in Pima cotton the stigmas project far beyond the summit of the staminal sheath, averaging in length, at 8 a. m., or about 1½ hours after the corolla has begun to open, 10 millimeters, or one-

[8] Darwin (*13*, p. 389), evidently having in mind plants on which numerous flowers are in anthesis at the same time, states "Insects usually search a large number of flowers on the same plant before they fly to another, and this is opposed to cross-fertilization."

[9] Knuth (*33*, p. 206). According to K. Schumann (*42*, p. 32) all Malvaceæ are protandrous.

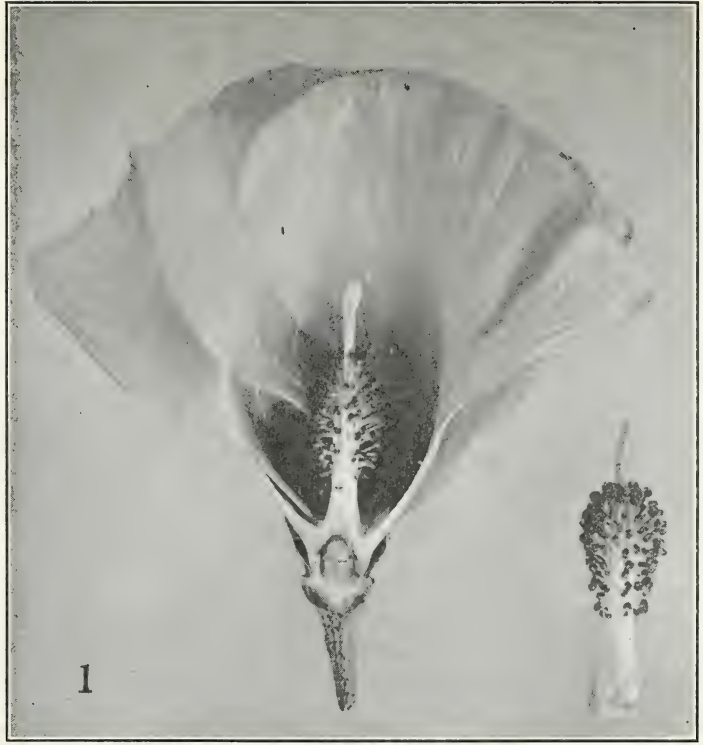


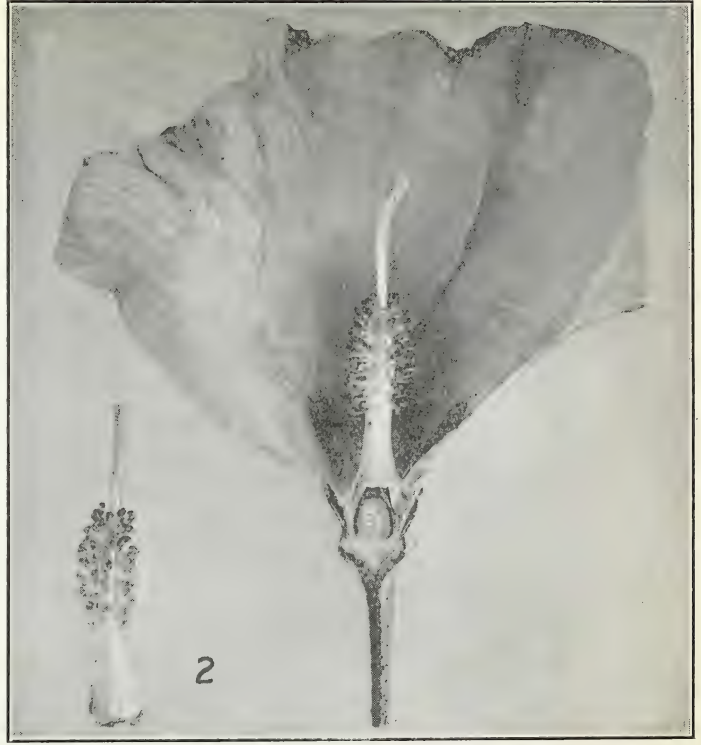


COTTON FLOWERS IN VERTICAL SECTION, SHOWING THE POSITIONS OF THE STIGMAS AND STAMENS.

FIG. 1.—Egyptian cotton, Pima variety, characterized by stigmas which greatly surpass the relatively short stamens and by having the basal portion of the stigmas closely screened by the uppermost stamens. FIG. 2.—Upland cotton, Acala variety, characterized by stigmas which barely surpass the relatively long stamens and by having no portion of the stigmas closely screened by the stamens.

Photographed by W. F. Gilpin.





COTTON FLOWERS IN VERTICAL SECTION.

FIG. 1.—Pima variety of Egyptian cotton. FIG. 2.—Sea island cotton, showing characters of the reproductive organs similar to those of Pima, except that the stigmas are longer and less densely screened at the base by the upper stamens.

Photographed by W. F. Gilpin.

third of the total length of the pistil exclusive of the ovary.[10] At the same hour the short uppermost stamens are found to extend on the average 2.5 millimeters above the summit of the staminal sheath. Consequently, at the time of opening of the corolla, approximately one-fourth of the total length of the stigmas is surrounded by the uppermost stamens. Owing to the density of the mass of surrounding stamens, this part of the stigmas probably is screened effectively against the access of foreign pollen. The erect or semierect position of the filaments of the upper stamens brings their anthers into close contact with the base of the stigmas, and automatic self-pollination is thus effected.

Trelease (*47*, p. 322), whose observations doubtless were made upon upland cottons, states:

> The reproductive organs are so placed that on the expansion of the corolla pollen has usually been deposited on the stigmas, self-fertilization being thus secured.

Robson (*41*) observes that "fertilization in the majority of cotton flowers is effected from the section of the stigma nearest the ovary."

The adaptation of the cotton flower both to self-fertilization and to cross-fertilization is described as follows by Kottur (*34*, pp. 52, 53):

> The entire surface of the style that projects beyond the staminal column is stigmatic; and this has been proved by cutting the stigma and fertilizing it only at the base. Again, in the majority of flowers the filaments of the upper anthers are sufficiently long to touch the base of the stigma. All these conditions are quite favorable for self-fertilization. The anthers are in contact with the stigma and they shed their pollen as soon as the flower opens. But, on the other hand, we have in most cottons a very attractive corolla. The quantity of honey and pollen in the flower is profuse and invites the insects that roam in search of them. All these favor natural crossings. We have thus one set of conditions favoring self-fertilization and another set favoring cross-fertilization; but the former occurs as a rule and the latter as an exception in all varieties of Indian cotton under observation at Dharwar.

The stamens of Pima cotton change their position very slightly, if at all, during the day. Observation as late as 3 p. m., when the corolla was beginning to wilt, showed the filaments of the uppermost stamens to be still erect and the anthers as though glued to the stigmas by the masses of extruded pollen.

In sea-island cotton (*Gossypium barbadense*) the positional relations of the reproductive organs (Pl. II, Fig. 2) are much the same as in Egyptian cotton, but the anthers do not form as dense a girdle around the base of the stigmas, which is probably somewhat more accessible to foreign pollen.

Most varieties of upland cotton (*Gossypium hirsutum*) are characterized by much shorter stigmas and by longer filaments of the stamens than in Egyptian cotton (Pl. I, Fig. 2). Measurements made in 1918 upon fully open flowers of the Pima (Egyptian) and Holdon (upland) varieties gave the means stated in Table 7, which show that in a typical upland cotton the stamens are much longer and the stigmas are much shorter, both absolutely and relatively, than in the Egyptian type as represented by the Pima variety.

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[10] Measurement of 100 Pima cotton flowers at 8 a. m. showed the mean length of the pistil from the summit of the ovary to be 30.5 ± 0.33 millimeters and the mean length of the stigmas (portion outside the staminal sheath) to be 10.2 ± 0.35 millimeters. The mean projection of the stamens above the summit of the sheath in the same flowers was 2.5 ± 0.16 millimeters.

TABLE 7.—*Measurements of the reproductive organs in Pima and Holdon cottons.*

Variety.	Number of plants on which flowers were measured.	Number of flowers measured.	Mean length of parts (millimeters).			Relative length of stigmas (percentage of pistil length).
			Stamens.[1]	Pistil.[2]	Stigmas.	
Pima (Egyptian)	21	83	5.0±0.01	36.3±0.29	9.8±0.21	27.0±0.45
Holdon (upland)	19	84	8.0± .03	26.5± .14	3.1± .08	11.7± .48
Differences			3.0± .03	9.8± .32	6.7± .22	15.3± .65

[1] Measured from the base of the filament to the apex of the anther. The average length of 10 stamens per flower was taken as the unit in computing these means. Stamens from near the middle of the staminal column were measured. The uppermost stamens are much shorter. Measurement in 1921 of 5 upper and 5 middle stamens per flower in 5 flowers, each from a different plant of Pima cotton, gave the following means: Uppermost stamens, 2.4±0.04 millimeters; middle stamens, 4.3±0.05 millimeters.

[2] Measured from the bottom of the corolla (hence somewhat below the summit of the ovary) to the apex of the stigmas.

Examination in 1920 of the flowers of 20 varieties of upland cotton growing at Sacaton, Ariz., showed that, shortly after the corolla begins to open, the upper stamens are erect. In 16 varieties they were from slightly shorter than to slightly longer than the stigmas, while in 4 varieties the stigmas exceeded the stamens by lengths not greater than 5 millimeters.[11] In most of the upland varieties, therefore, the whole or the greater part of the length of the stigmas is surrounded by the stamens, and the erect position of the latter during the first hour or so after the opening of the flower brings the anthers into contact with the stigmatic surface. Unlike the condition in Egyptian cotton, there is a limited power of movement, for later in the day the filaments become more nearly horizontal. It was observed in 1921 that in the Acala variety at 3 p. m. most of the uppermost filaments diverged at angles of 20° to 45°, although even at this hour occasional anthers remained in contact with the stigmas. The entire length of the stigmas in upland cottons is at all times, however, much more accessible to foreign pollen than is the interstamen section of the Egyptian stigmas.

If one overlooks the receptive character of the entire surface of the pistil outside the staminal sheath and the possibility of a high degree of fertilization by self-pollen automatically discharged upon the basal portion of the stigmas, the assumption is likely to be made that varieties of cotton having long stigmas are not well adapted to self-fertilization. Meade (*40*) drew this conclusion from the results of an experiment with upland cottons performed by him at San Antonio, Tex., in which flowers of a short-style variety (Acala) and of a longer styled variety (Durango) were artificially pollinated, the stigmas having been thoroughly smeared with their own pollen. Comparing these artificially pollinated flowers with naturally pollinated flowers of the same varieties in respect to the percentages of bolls set, the variety having long stigmas showed a mean increase of 11.0±2.2 per cent from artificial pollination, as compared with an increase of only 5.3±2.4 per cent in the variety having short stigmas,

[11] It is possible that the stigmas were abnormally short in some of the upland cottons grown at Sacaton in 1920, among which were several of the long-staple varieties. According to Meade (*40*) in many of the long-staple upland varieties the stigmas often exceed the anthers by 15 millimeters.

but the difference between the increases in the two cases was less than twice its probable error.

The converse proposition, that the ovules of flowers with short stigmas are less likely to be cross-fertilized than those of flowers having long stigmas, would seem to be self-evident. It was not borne out, however, by the results of an experiment performed by Balls (*8*, pp. 118, 119) who compared two strains derived from an Egyptian-upland cross, one of which had the stigmas so short as to be surpassed by the uppermost anthers, while the other had stigmas which greatly surpassed the anthers. No difference was found between the two strains in the percentage of hybrids resulting from natural cross-pollination. Apparently in this case short stigmas offered no effective obstacle to the access of foreign pollen. It is of interest in this connection to note that the Lone Star variety of upland cotton, in which the stigmas normally are exceeded by the upper stamens, produced at Sacaton, Ariz., 5 per cent of vicinists when grown in a row adjacent to a row of Pima (see Table 1, p. 5). The probable explanation is that both in the hybrids compared by Balls and in the Lone Star variety the density of the screen formed by the stamens was not sufficient to protect the short stigmas from access of foreign pollen.

FLOWER STRUCTURE IN RELATION TO CROSS-FERTILIZATION.

In Pima cotton the deposition of foreign pollen upon the basal portion of the stigmas presumably is prevented by the density of the surrounding girdle of stamens (Pl. I, Fig. 1; Pl. II, Fig. 1). An experiment was performed in 1919 with the object of determining the effectiveness of this protection. The material consisted of a row of Pima plants having on one side a row of the Holdon variety and on the other side a row of the Lone Star variety, the populations being the same as in the vicinism experiment (Table 1). Flower buds of all three varieties were opened before anthesis, and the extrastaminal portion of the stigmas, if any, was excised, after which the flowers were left exposed to the visits of insects. In the Lone Star variety the stigmas usually are exceeded by the stamens; hence little or no excision was necessary in this case. In the Holdon variety the portion excised was much shorter than in the case of Pima.

The seed produced by the treated flowers of the three varieties was planted in 1920. The population from seed borne by the Pima plants was much larger than the upland populations, for the reason that the proportion of the treated flowers which failed to set bolls was much larger in the upland cottons[12] than in Pima, and the quantity of seed produced was consequently much greater in the latter case. Early in July, when the plants were well enough developed to show their characters clearly, counts were made of the number of hybrids in the three populations. The rows were not thinned, so that all plants which survived the germination and seedling stages were counted.[13] The results are given in Table 8.

[12] This does not indicate that the excision of the extrastaminal part of the stigmas had been more injurious to the upland than to the Egyptian flowers, as the rate of boll shedding at Sacaton, Ariz., is always much higher with upland than with Egyptian cotton.

[13] Data given on page 5 indicate that there had been no natural selection in the earlier stages of growth which would affect the percentages of hybrids.

TABLE 8.—*First-generation hybrids in progenies grown at Sacaton, Ariz., in 1920, from seed produced by flowers the stigmas of which had been excised in the bud at the level of the uppermost stamens.*

Variety of which seed was produced by excised flowers.	Total plants.	F_1 hybrids.	
		Number.	Per cent.
Pima (Egyptian)	172	0	0
Lone Star (upland)	19	9	47.3±7.7
Holdon (upland)	54	1	1.8±1.2

Reference to Table 1 shows that a population grown from seeds produced by unmutilated naturally pollinated flowers of the Pima plants used in the present experiment contained approximately 3 per cent of hybrids, while the data given in Table 8 show that excision of the extrastaminal portion of the stigmas had prevented cross-fertilization of the Pima flowers. This might have been explained on the ground that the removal of a portion of the corolla in the process of excising the stigmas had rendered the flowers unattractive to insects, were it not for the fact that cross-fertilization occurred in similarly treated flowers of the two upland varieties. It seems probable, therefore, that in Pima cotton the basal portion of the stigmas is effectively screened by the surrounding stamens against the access of foreign pollen, whereas in upland cottons no portion of the stigmas is inaccessible to such pollen.

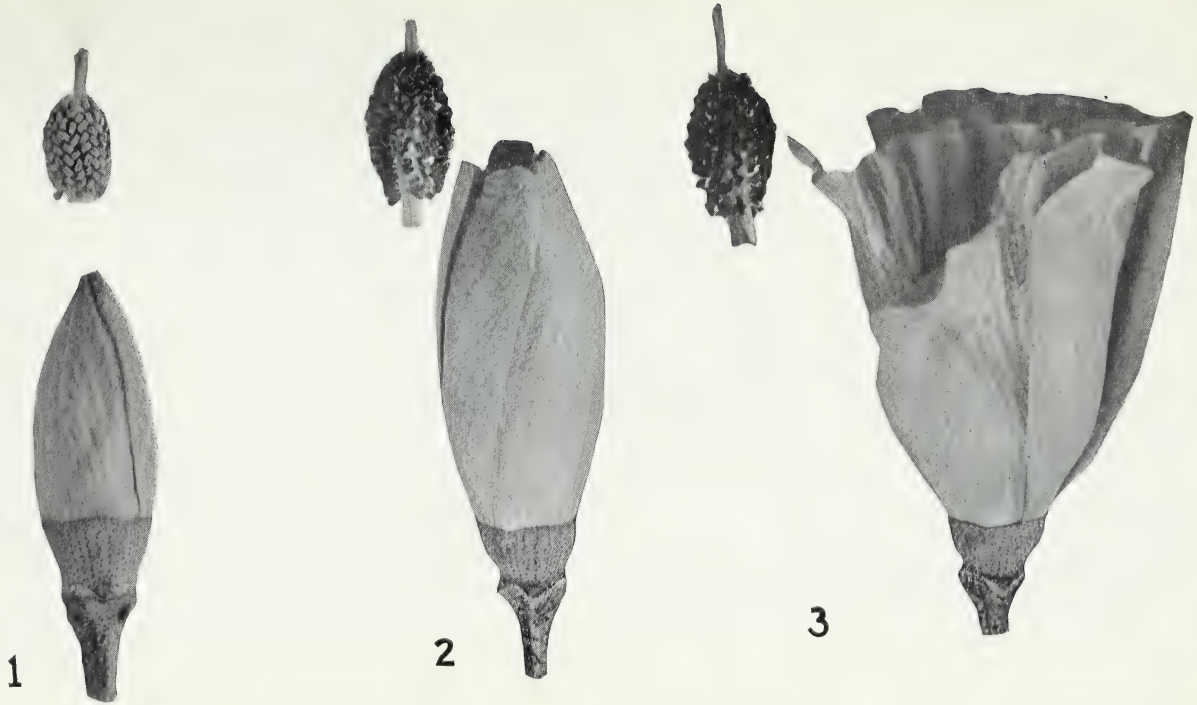
An anomalous result of the experiment is the much higher percentage of hybrids in the population derived from treated flowers of the Lone Star variety than in the corresponding Holdon population, whereas in the vicinism experiment, involving untreated naturally pollinated flowers on the same plants (Table 1), Holdon yielded more than twice as high a percentage of hybrids as Lone Star, and the difference was five times its probable error.

ONTOGENY OF THE FLOWER IN RELATION TO POLLINATION.

Only the last stages in the ontogeny of the flower are of importance in relation to pollination. The time and rate of opening of corolla and anthers, the condition of the pollen from shortly before the flower opens until it has begun to wilt, and the duration of receptivity of the stigmas will be considered in this connection. The final stages in the development of the flower are illustrated in Plate III.

OPENING OF THE COROLLA.

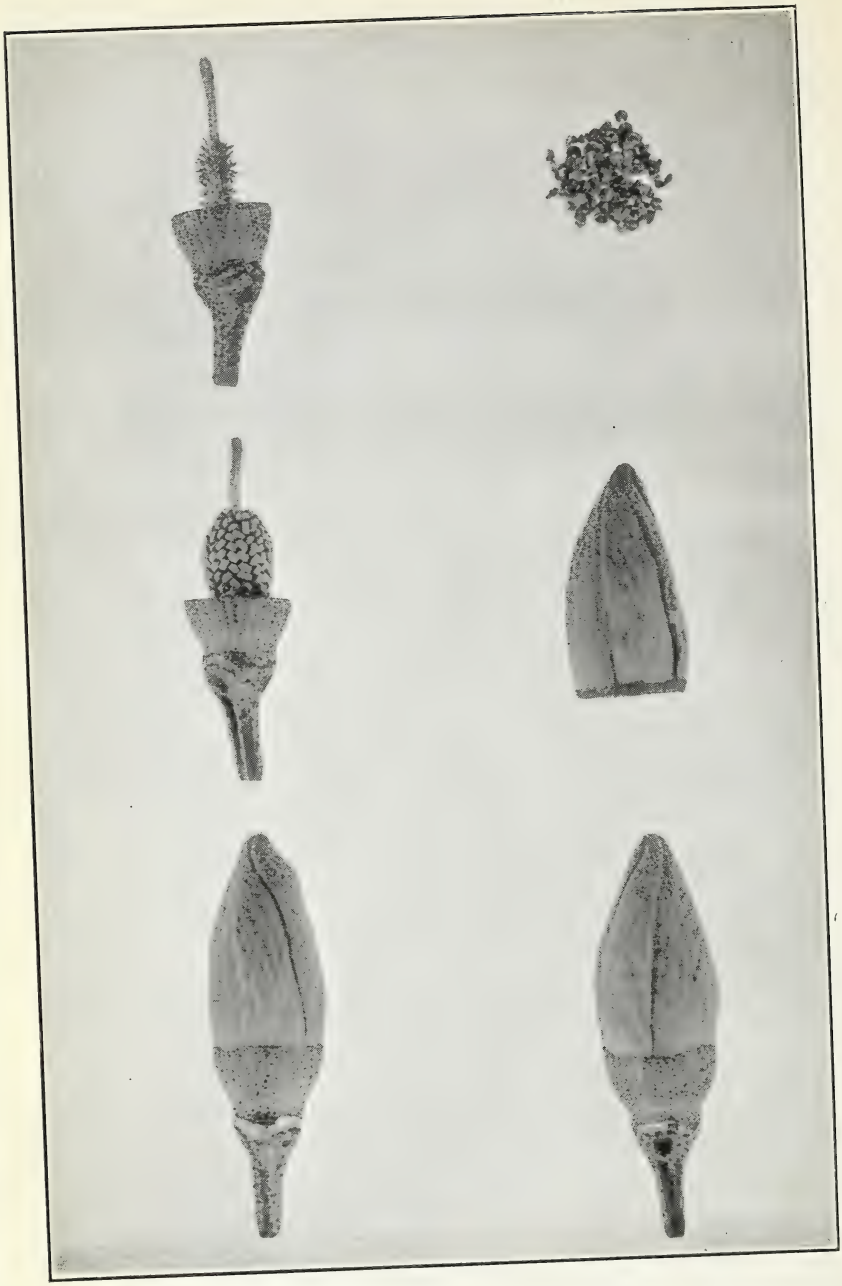
The bud remains tightly closed during the night preceding anthesis, the petals beginning to separate at the apex usually about an hour after sunrise. During the next hour or so the opening of the corolla proceeds slowly, but thereafter the aperture widens rapidly, with a slowing down in the rate shortly before the maximum diameter is attained. Accurate data as to the rate of opening were obtained from an experiment performed in 1919. During three periods of five days each (July 29 to August 2, August 18 to August 22, and September 11 to September 15) 20 flowers were tagged daily, and the aperture of the corolla was measured at half-hour intervals. The mean diameter of the aperture for each half hour is stated in Table



FLOWERS OF PIMA COTTON IN LATE STAGES OF DEVELOPMENT.

FIG. 1.—At 5 p. m. of the day preceding anthesis, showing the corolla and anthers still tightly closed. This is the condition of the flower buds at the time emasculation is performed. FIG. 2.—At 7.30 a. m. of the day of anthesis, showing the corolla in an early stage of expansion and the anthers partly open. FIG. 3.—At 9.30 a. m., showing the corolla almost fully expanded and the anthers wide open.

Photographed by W. F. Gilpin.



FLOWER BUDS OF PIMA COTTON.

The successive stages of the process of emasculation during the evening preceding anthesis: Intact buds (bottom); bud with corolla removed, showing the tightly closed anthers (middle); anthers removed, flower ready for pollination (top).

Photographed by W. F. Gilpin.

9, the probable error having been computed from the departures of the daily means of 20 flowers from the mean of the daily means. The half-hour means for the day when the opening was most rapid

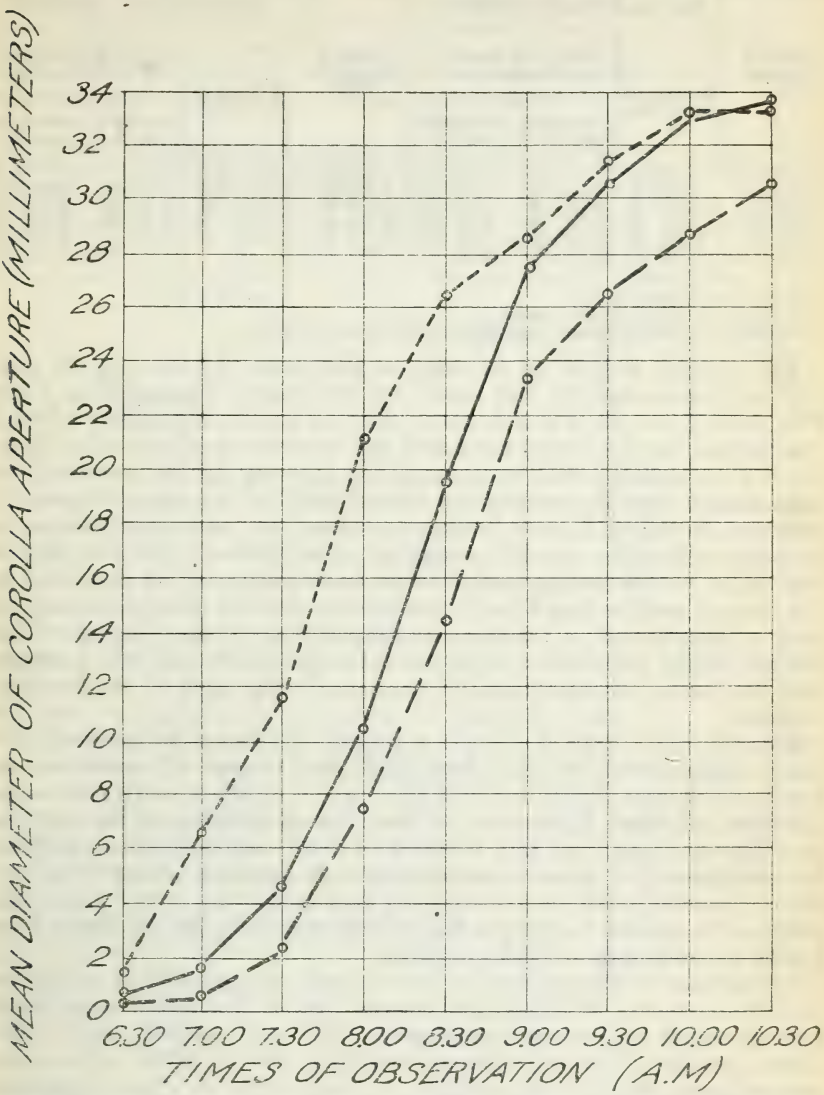


FIG. 2.—Average, maximum, and minimum rates of expansion of the corolla of Pima cotton, as indicated by the mean aperture of the corolla at half-hour intervals during the morning, this having been determined by measurement of 20 flowers on each of 15 days during the period from July 29 to September 15, 1919. The mean hour of sunrise during this period was 5.26. The dotted and broken lines indicate the rates on the days when expansion was most rapid and least rapid, respectively, and the solid line indicates the average rate of expansion for all 15 days.

and for the day when it was least rapid are also given in the table. The average, maximum, and minimum rates of expansion are shown graphically in Figure 2.

TABLE 9.—*Measurements of the aperture of the corolla of Pima cotton at successive half-hour intervals during three periods of five days each in 1919.*

Hour of measurement.	Daily mean diameter (millimeters).			Hour of measurement.	Daily mean diameter (millimeters).		
	Average for the 15 days.	For the day when the rate of opening was—			Average for the 15 days.	For the day when the rate of opening was—	
		Most rapid (Aug. 18).[1]	Least rapid (Aug. 2).[2]			Most rapid (Aug. 18).[1]	Least rapid (Aug. 2).[2]
6.30 a. m.....	0.6±0.03	1.3±0.13	0.4±0.04	9.00 a. m.....	27.3±0.60	28.7±0.58	23.3±0.66
7.00 a. m.....	1.6± .18	6.6± .48	.7± .12	9.30 a. m.....	30.7± .40	31.2± .53	26.7± .63
7.30 a. m.....	4.7± .39	11.7± .42	2.2± .38	10.00 a. m.....	32.9± .47	33.2± .55	28.8± .55
8.00 a. m.....	10.6± .69	21.0± .67	7.6± .75	10.30 a. m.....	33.7± .43	33.2± .55	30.3± .65
8.30 a. m.....	19.6± .90	26.3± .56	14.3± .85	11.00 a. m.....	[3]	33.2± .55	31.5± .71

[1] Sky clear at and after sunrise.
[2] Sky partly cloudy at and after sunrise.
[3] Omitted in the general average because not determined on several days.

The time of sunrise in Arizona in 1919 was 5.11 on July 29 and 5.42 on September 15, the mean for the period having been 5.26. The data given in Table 9 show that as a rule expansion of the corolla had barely commenced at 6.30, or about one hour after sunrise. It is evident that in general the opening of the corolla proceeded most rapidly during the hour 8 to 9, the average increase in aperture during this hour having amounted to one-half of the mean diameter when the corolla ceased to open farther. As the period during which the measurements were made comprised 48 days and as the time of sunrise was 31 minutes later at the end than at the beginning of this period, a progressive retardation of the opening of the corolla might have been expected. In fact, however, the average rate of opening was practically the same during each of the five-day periods.

Records were kept for each morning of the experiment of the shade temperature, relative humidity, and degree of cloudiness at hourly intervals beginning at 6.30 a. m., the object having been to ascertain whether differences in the rate of opening of the corolla on different days bore any relation to these meteorological factors. No evidence of a general correlation was detected, except that on cloudy mornings the rate of opening was somewhat slower and more gradual, the curve showing a less abrupt rise between the hours 7 to 9 than on mornings of full sunshine.

Simultaneous observations of the rate of opening of the corolla in the Pima variety of Egyptian cotton and in the Acala variety of upland cotton on several mornings in July and August, 1920, indicated that as a rule the opening begins a few minutes earlier and proceeds somewhat more rapidly in Acala than in Pima, notwithstanding the fact that the Pima flowers, which are borne on longer fruiting branches, are more exposed than the Acala flowers to the early rays of the sun.

Observations made in 1921 afforded data as to the relative earliness of opening of the corollas of Pima and of upland varieties, the first appearance of an aperture having been taken as the criterion. On August 11, 50 flowers of Pima and 24 flowers of King (upland) were examined. Of these flowers 60 per cent showed an aperture as early as 6.30 in the case of King, but not until 7.15 in the case of

Pima; 90 per cent showed an aperture at 7.05 in the case of King, but not until 8.05 in the case of Pima. Similar observations on August 12 on 50 flowers each of Pima and of the Durango, Acala, and Lone Star varieties of upland cotton indicated, on the contrary, the more rapid appearance of an aperture in Pima than in the upland varieties. The hours at which an aperture had appeared in 50 and in 90 per cent of the flowers examined are shown in Table 10.

TABLE 10.—*First appearance of an aperture in the corollas of 50 flowers of each of four varieties of cotton grown at Sacaton, Ariz., as observed on August 12, 1921.*

Aperture present—	Time of opening (a. m.).			
	Pima (Egyptian).	Upland varieties.		
		Durango.	Acala.	Lone star.
In 50 per cent of the flowers	7.15	7.40	7.50	.....
In 90 per cent of the flowers	7.50	8.10	8.25	8.55

While the several observations gave contradictory results as to the relative earliness of the first appearance of an aperture in Pima and in upland varieties, it appears that the further expansion of the corolla proceeds more rapidly in upland than in Pima. Comparing the Pima and Acala varieties it was observed that in the former expansion begins with a very minute aperture at the apex of the bud, which enlarges gradually, whereas in Acala the initial aperture is larger and the petals separate much more rapidly. The greater length of the Pima petals and the fact that they are more tightly wrapped in the bud probably explain this difference.

The flower of both Egyptian and upland cottons is of brief duration. On sunny days in July and August the corolla begins to wilt and change color by midafternoon, and before sunset the wilting has proceeded so far that the corolla is closed or nearly so and the pistil is becoming flaccid. Abscission of the style in the Pima variety normally takes place within 36 hours after the beginning of anthesis. Observations on 50 Pima flowers in 1922 showed that in every case the style had separated from the ovary by 2 p. m. of the day following anthesis, or 31 hours after the commencement of anthesis. The mean number of hours from the commencement of anthesis to the abscission of the style was 29 ± 0.08.

OPENING OF THE ANTHERS AND DISCHARGE OF POLLEN.

Examination of flower buds of Pima cotton late in the afternoon preceding the opening of the corolla (Pl. III, Fig. 1) shows, as a rule, the anthers tightly closed and the pistil free from pollen grains. At this stage the pollen can not be extracted easily from the anthers. Occasional flowers have been observed in which a few of the anthers were open sufficiently in the evening to show the pollen grains, but in none of these cases was pollen found upon the stigmas under conditions making it certain that the discharge had not taken place as a result of rupturing the anthers in the process of cutting away the corolla. In the early morning, however, when the corolla is

still closed or is open at the apex to the extent of not more than 1 or 2 millimeters, as is usually the case in July and August up to about 7 a. m., the anthers are found to be partly open, so as to expose the pollen. The rapidity with which both corolla and anthers open depends to some extent upon the position of the flower on the plant, which determines how early it is exposed to the rays of the sun. The condition of the sky also is doubtless a factor in the earliness of opening.

On July 25, 1920, during the half hour from 5.30 to 6 a. m. 25 Pima flowers which had the corolla still closed, although in some cases the petals were beginning to loosen at the tip, showed the anthers to be partly open in all of the flowers. Pollen grains in greater or less number were already present on the interstamen region of the stigmas in all but one flower, but some of this pollen may have been deposited in the act of opening the bud. That this was probably the case is indicated by further observations during the same summer, in which extreme care was taken to avoid the discharge of pollen upon the stigmas while opening the bud. Ten flowers were examined at about 7 a. m. on each of six days during the period from August 3 to August 14. At the time of observation the corolla was closed or was open to an extent of not more than 1 or 2 millimeters, while the anthers were at least half open and were extruding pollen. Pollen was found upon the interstamen region of the stigmas in about half of the flowers, but the number of grains there present was very small, frequently not exceeding one or two.

When the natural opening of the corolla is delayed the discharge of pollen also is retarded.[14] On July 25, 1917, 10 closed or nearly closed flowers were examined at 8 a. m. (hence nearly three hours after sunrise), and four of these had no pollen on the stigmas, even on the portion surrounded by the uppermost anthers. Examination, on July 30, 1920, of a few buds which were still closed at 8 a. m. showed that self pollen was just beginning to be deposited upon the stigmas in appreciable quantity. On August 19, 1921, fully 90 per cent of the flowers were open sufficiently between 7.30 and 8 a. m. to admit insects. Of the belated buds, which were either tightly closed or were just beginning to loosen at the tip, 20 were opened during this half hour, taking every precaution to avoid further discharge of pollen, and the number of grains present on the stigmas was determined as accurately as could be done without touching the anthers. The counts showed numbers of grains present on the lower half of the stigmas as follows: In 8 flowers, 6 grains or fewer; in 5 flowers, 6 to 12 grains; in 7 flowers, more than 12 grains. Of the buds examined 65 per cent had no more than a dozen grains of pollen present on the stigmas.

In upland cottons the opening of the anthers may or may not precede that of the corolla. Five flowers each of some 20 upland varieties were examined at Sacaton, Ariz., in 1920. In six varieties some of the flowers had the anthers still closed after the corolla had begun to expand. In the other varieties the opening of the anthers was keeping pace with the expansion of the corolla in most of the flowers. Examination of 10 closed or barely opening flower buds

[14] Cook (*11*, p. 204) states that "in cool moist weather the anthers sometimes fail to open, so that no pollen is available." An instance of complete failure to set bolls from this cause was observed in Guatemala.

of the Acala variety of upland cotton, on each of six dates from August 3 to August 14, 1920, showed that at about 7 a. m. discharge of pollen had begun in only one-third of the flowers. A comparison was made of the relative rates of opening of the corolla and anthers in Pima and Acala cottons on August 9, 1920. It was observed at 7 a. m. that in Pima the corollas were open only 1 or 2 millimeters, but the anthers were well open; while on near-by Acala plants the corollas were open from 5 to 10 millimeters, but the anthers were still closed or were just beginning to split.

Further observations were made at Sacaton, Ariz., in 1921. At 7.30 to 8 o'clock on the cool, cloudy morning of July 27, upland flowers of which the corollas were already open to an extent of 5 to 10 millimeters had the anthers in most cases either still closed or split only sufficiently to disclose but not to discharge the pollen grains. Only one of the eight varieties examined showed the discharge of pollen in some of the flowers before the corolla had commenced to expand, while in all closed buds of Pima cotton, which were examined at the same time, the anthers were wide open, and in many of them the discharge of pollen upon the stigmas had begun. On the other hand, on August 12 at 7.30 to 7.45 a. m. examination of closed buds of the Acala variety showed that the anthers were partly open in all cases and that a few pollen grains were present on the stigmas in 7 of the 10 buds examined.

Observations upon upland cotton have shown that dehiscence of the anthers and discharge of pollen before the petals have begun to unfold are more likely to occur in belated flowers than in flowers which have not been retarded in their opening. Thus, on August 9, 1921, a warm, sunny morning, when most of the flowers of upland varieties were already open at 8.15 o'clock, approximately two-thirds of the buds which still remained closed had the anthers partly open. In many of these buds a few grains of self pollen were already present on the stigmas. Closed buds of Pima cotton examined during the same half hour had the anthers much more fully open than in the upland varieties, and in nearly every case the stigmas had received self pollen in greater or less quantity. On the following morning, with similar weather conditions, observations were made on the Lone Star and Acala varieties of upland cotton and on Pima cotton during the half hour from 8.10 to 8.40, when most of the flowers of the three varieties were partly open. Of the still closed buds of Lone Star, 20 were opened carefully, and 9 of these were found to have the anthers partly open and a few grains of pollen on the stigmas. In the remaining 11 buds the anthers were still closed or were beginning to split but were not yet discharging pollen. Of six closed flowers of the Acala variety, four had the anthers partly open and a few grains of pollen present on the stigmas. Ten unexpanded Pima flowers had the anthers much wider open than in the upland varieties, and in most but not all of these a little pollen was present on the stigmas.

It may be concluded from these observations on the comparative rate of opening of the corolla and anthers in Pima and upland cottons that in Pima the opening of the anthers and the discharge of pollen somewhat precede the expansion of the corolla, while in upland as a rule the corolla and anthers begin to open almost simultaneously. In case the opening of the upland corolla has been retarded, how-

ever, the anthers often begin to open and to discharge pollen before the petals commence to unfold. It seems clear that even in Pima cotton no considerable quantity of pollen ordinarily is deposited upon the stigmas before the expansion of the corolla has begun.

Other investigators have noted that different species of Gossypium differ in the rate of opening of the anthers. A statement by Fyson (*18*, p. 5) implies that in India the anthers of American upland cotton (*Gossypium hirsutum*) open and discharge their pollen earlier than do anthers of Asiatic species (*G. herbaceum*, etc.). Smith (*44*), in the West Indies, observed that sea-island cotton (*G. barbadense*) opens its anthers earlier in the day than does a native cotton of the American upland type.

VIABILITY OF THE POLLEN IN DIFFERENT STAGES OF DEVELOPMENT.

The viability of the pollen during the hours immediately preceding and following the opening of the corolla is of interest in relation to the phenomena of pollination. The rapidity and completeness with which the pollen grains eject their contents at different stages in the ontogeny of the flower were tested by immersing them in a 5 per cent aqueous solution of cane sugar, although apparently ejection takes place with equal readiness in water.

The discharge of protoplasm by the pollen grain in these media takes place in the manner described as "pseudogermination" by Andronescu (*4*), the contents being ejected with explosive suddenness in a very long slender thread, which immediately becomes twisted into a tangled spiral. Andronescu's illustration of the process in Zea (*4*, pl. 2) represents very well the phenomenon as it occurs in Gossypium. It is uncertain in what degree the rate of pseudogermination at different hours of the day is correlated with that of normal germination upon the stigmas. It will be shown, however, that in cotton little or no pseudogermination takes place in the evening preceding the opening of the corolla and that it increases in rapidity and completeness during the following morning, reaching a maximum intensity at noon and then gradually declining to a very low minimum long before sunset. It seems at least probable that normal germination follows a similar course and that the vigor of pseudogermination is indicative of the viability of the pollen (*4*, p. 16). The phenomenon will be referred to in this bulletin as "ejection," thus avoiding the cumbersome term "pseudogermination."

Observations were made with a binocular microscope. The pollen was immersed in the sugar solution as soon as possible after detaching the flower from the plant. The criteria of viability used were (1) the number of seconds after immersion until active ejection ceased and (2) the percentage of the total number of grains in the field of the microscope which discharged their protoplasm during the period of active ejection. An "index of viability," which integrates rapidity and completeness of ejection at different hours, was obtained by dividing the percentage of the total grains ejected by the number of seconds required to complete active ejection and multiplying the quotient by 100.

The condition of the pollen on the day preceding anthesis will be considered first. Pollen from Pima buds was collected on several

occasions at from 3 to 6 p. m., and its reaction in a solution of cane sugar was observed. After immersion during one to five minutes a small percentage of the grains ruptured and their contents oozed out slowly, the phenomenon having been very different from the explosive ejection of a long thread which was observed in pollen grains collected in the morning from open flowers.

The viability of the pollen of Pima cotton during the day of anthesis was tested on July 26, 1917, and on July 25 and August 5, 1919. As the results show close agreement, only those of August 5 will be considered in detail, parallel tests with the Durango variety of upland cotton having been made on that date. Pollen of each variety was collected at half-hour intervals from 6 a. m. (hence before the corollas had opened) until 10.30 a. m. and at intervals of one hour thereafter until 3.30 p. m., with a final collection at 5.30 p. m. Shortly before the first collection was made the sky was cloudy, but during the remainder of the day there was

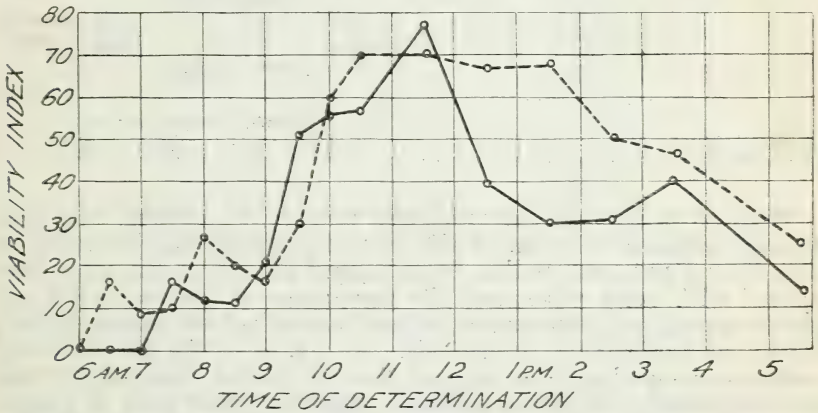


FIG. 3.—Indexes of viability of the pollen of Pima (Egyptian) and of Durango (upland) cotton at successive time intervals during the day of anthesis. The curve for Pima is indicated by a solid line and that for Durango by a dotted line. Both curves show a low viability early in the morning, a rapid increase beginning at 8.30 or 9 o'clock, and a gradual decline after midday.

full sunshine. The tests were made upon one flower of each variety up to 10.30 a. m. and thereafter on two flowers of each variety, the average of the viability indexes of the two flowers having been used in plotting the curves.

Endeavor was made to select only flowers which were so located on the plant as to have been exposed to full sunlight up to the time of collection. This object was realized in the case of Pima but not in the case of Durango, owing to the limited number of flowers available. However, no flowers of Durango were taken later in the day which had not been so exposed during several hours, and the earliest flowers in the most exposed situations were selected. The Durango anthers tended to become exhausted of pollen earlier in the day than the Pima anthers, probably because the shallow, flaring corolla of upland cotton attracts more of the large pollen-carrying insects.

The indexes of viability of the two varieties at different hours are shown by curves in Figure 3. The percentage of grains ejected, one

of the factors in computing the index was merely estimated, except in a few cases when the total number of pollen grains in the field of the microscope was small. The more rapid decline in viability during the afternoon shown by the Pima pollen was probably caused by the fact, already noted, that the flowers of this variety were more exposed to the direct rays of the sun than the Durango flowers.

Pollen has been found to retain its viability much longer in flowers which have been inclosed in paper bags to prevent cross-pollination than in open flowers. On July 26, 1917, buds due to open that morning were bagged at 6 a. m., and the viability of the pollen was tested in sugar solution at 6 p. m. of the same day and at 6 a. m. of the day following. The results are given in Table 11.

TABLE 11.—*Prolongation of the viability of the pollen in cotton flowers bagged at 6 a. m. July 26.*

Hour of testing.	Number of flowers.	After immersion until ejection—		Estimated ejection.	Viability index.
		Began.	Ceased actively.		
		Seconds.	*Seconds.*	*Per cent.*	
6 p. m., July 26	2	37	105	75	71
6 a. m., July 27	1	35	270	40	15

Whereas in the case of unbagged flowers the proportional ejection of pollen at 6 p. m. of the day of anthesis was estimated at only 3 or 4 per cent, pollen from bagged flowers at the same hour ejected with great vigor, and the percentage of grains ejected was almost as high as in the case of pollen from uninclosed flowers shortly before noon of the day of anthesis. At 6 a. m. of the day following anthesis the pollen from bagged flowers ejected more slowly and less completely. It seems probable, therefore, that even in bagged flowers the pollen loses its viability during the day following anthesis.[15]

It may be deduced from the curves shown in Figure 3, which are based upon an index integrating the percentage of pollen grains which eject their contents and the rapidity with which ejection is completed, that under conditions at Sacaton, Ariz., the viability of the pollen of Pima (Egyptian) and of Durango (upland) cotton is low during the early morning hours, begins to increase rapidly at about 9 o'clock, and begins to decline at or shortly after midday. If the index of viability based upon the rapidity and completeness of ejection in a sugar solution indicates the capacity for normal germination, it would be concluded that pollen which reaches the stigmas before 8 or 9 a. m. will germinate more slowly and less completely than pollen which arrives later in the morning. It should be noted, however, that while at earlier hours a much longer time was required for the ejection to take place, the percentages of the total number of grains which finally ejected their contents were in some cases relatively high. Thus, in the case of Pima cotton, ejection

[15] Pollen longevity in the snapdragon and in maize is the subject of a recent publication by H. E. Knowlton (32), who summarizes (p. 755–759) the results of other investigators with various plants and points out (p. 786) that pollen may retain its capacity to germinate when no longer able to effect fertilization.

finally took place in about 70 per cent of the grains collected at 6.30 a. m. on July 26, 1917, and in about 65 per cent of those collected at 6 a. m. on July 25, 1919. A test of Durango pollen on August 5, 1919, showed ejection at 6.30 a. m. in about 75 per cent of the total number of grains. It also seems probable that pollen discharged at an early hour may continue to mature after it has reached the stigmas.

DEGREE OF MATURITY OF POLLEN AS AFFECTING FERTILIZATION.

An experiment was performed in 1921 to ascertain whether fertilization can be effected by immature pollen placed upon the stigmas many hours in advance of the time of anthesis. Pima flower buds were emasculated in the evening, pollen squeezed from the anthers of the same flower was placed upon the stigmas, and the flowers were kept inclosed in bags until the stigmas had withered and there was no longer danger of accidental cross-fertilization. Only 1 of 25 flowers thus treated produced a boll which reached maturity. This boll contained five ripe seeds. A second boll was retained longer than 10 days but finally dropped, the exact date not having been ascertained. The remaining 23 flowers shed their undeveloped bolls within 10 days of the date of pollination, this being apparently the average number of days from anthesis to shedding for Pima cotton in Arizona (*31*), p. 15). The results of this experiment are of practical interest as showing that when flowers are emasculated the evening before anthesis for the purpose of making hybrids, every precaution should be used to prevent self pollen from reaching the stigmas, fertilization with such pollen being possible, although evidently not frequent.

The methods used in emasculating and bagging flowers in this and in experiments subsequently described are illustrated in Plates IV and V.

In order to ascertain whether fertilization is affected by deferring pollination several hours after it would take place normally, 240 Pima flower buds were emasculated late in the afternoon preceding anthesis during the period from July 22 to August 2, 1921, 20 buds having been treated on each day of the experiment. Other flower buds were bagged at the same time to supply the pollen required. Half of the flowers were pollinated at 8 o'clock the following morning and the others at about 5 p. m. In open flowers the anthers would have been practically empty of pollen, and the stigmas would have been losing their turgidity at the latter hour, but it has been shown that bagging tends to prolong the freshness of the flower.

The number of bolls which matured and the number of seeds in each boll were determined, and from these data were computed the percentage of bolls matured and the mean number of seeds per boll, as stated in Table 12. The difference in the percentage of bolls matured was in favor of the deferred pollination, but was less than three times the probable error. On the other hand, early pollination yielded a somewhat higher mean number of seeds per boll, and this difference was approximately three times its probable error. It may be concluded that in bagged flowers the pollen retains its ability to effect fertilization practically unimpaired up to 5 p. m. of the day of anthesis.

TABLE 12.—*Comparison of morning and evening pollination of cotton flowers, showing the percentage of bolls matured and the mean number of seeds per boll.*

Time of pollination.	Flowers treated.	Percentage of bolls matured.	Mean number of seeds per boll.
Flowers pollinated at 8 a. m.	120	89.2±1.93	14.5±0.23
Flowers pollinated at 5 p. m.	120	95.0±1.35	13.4± .26
Difference		5.8±2.35	1.1± .35

Another experiment was performed in 1921 with the object of ascertaining whether the pollen in bagged flowers remains viable as long as 26 hours after anthesis would have begun under normal conditions. Pima flower buds were emasculated in the evening of August 5 and pollinated on August 6. Of the 100 buds emasculated 50 were pollinated at 1 p. m. with pollen from flowers which had been bagged on August 5 at the time the emasculation was done. The other 50 were pollinated at 9 a. m. with pollen from flowers bagged in the bud on August 4 which at the time of pollination were about 26 hours past the normal time of the beginning of anthesis. In these old flowers when collected for use in pollination, the petals were wilted and the pollen was very loose in the anthers. The relative fertilization obtained from the two pollinations, as shown in Table 13, indicates that although the flowers had been protected by inclosure in bags, much of the pollen had lost its ability to effect fertilization 26 hours after the normal time of the beginning of anthesis.

TABLE 13.—*Results obtained by pollination with old and with fresh pollen, showing the percentage of bolls matured and the mean number of seeds per boll.*

Pollination with pollen from flowers in which anthesis normally would have begun—	Flowers treated.	Percentage of bolls matured.	Mean number of seeds per boll.
6 hours previously (fresh pollen)	49	85.7±3.44	12.6±0.41
26 hours previously (old pollen)	50	38.0±4.63	6.4± .58

DURATION OF THE RECEPTIVITY OF THE STIGMAS.

It has been mentioned that in uninclosed cotton flowers at Sacaton, Ariz., the stigmas show a perceptible loss of turgor before sunset of the day of anthesis. The results of the experiment summarized in Table 12 indicate, however, that when the flowers have been protected by bagging, the stigmas show no appreciable loss of receptivity, as measured by the degree of fertilization attainable, as late as 5 p. m. In order to determine whether the stigmas retain their receptivity for a still longer period when the flowers are inclosed, 100 Pima flower buds were emasculated and bagged during the evening of August 3, 1921, and were cross-pollinated with fresh pollen from Pima plants, half of them at 1 p. m. of the day following emasculation and half at 8 a. m. of the second day. It proved somewhat difficult to extract pollen from the anthers of the

INCLOSING A COTTON FLOWER IN A PAPER BAG TO PREVENT ACCIDENTAL CROSS-POLLINATION.

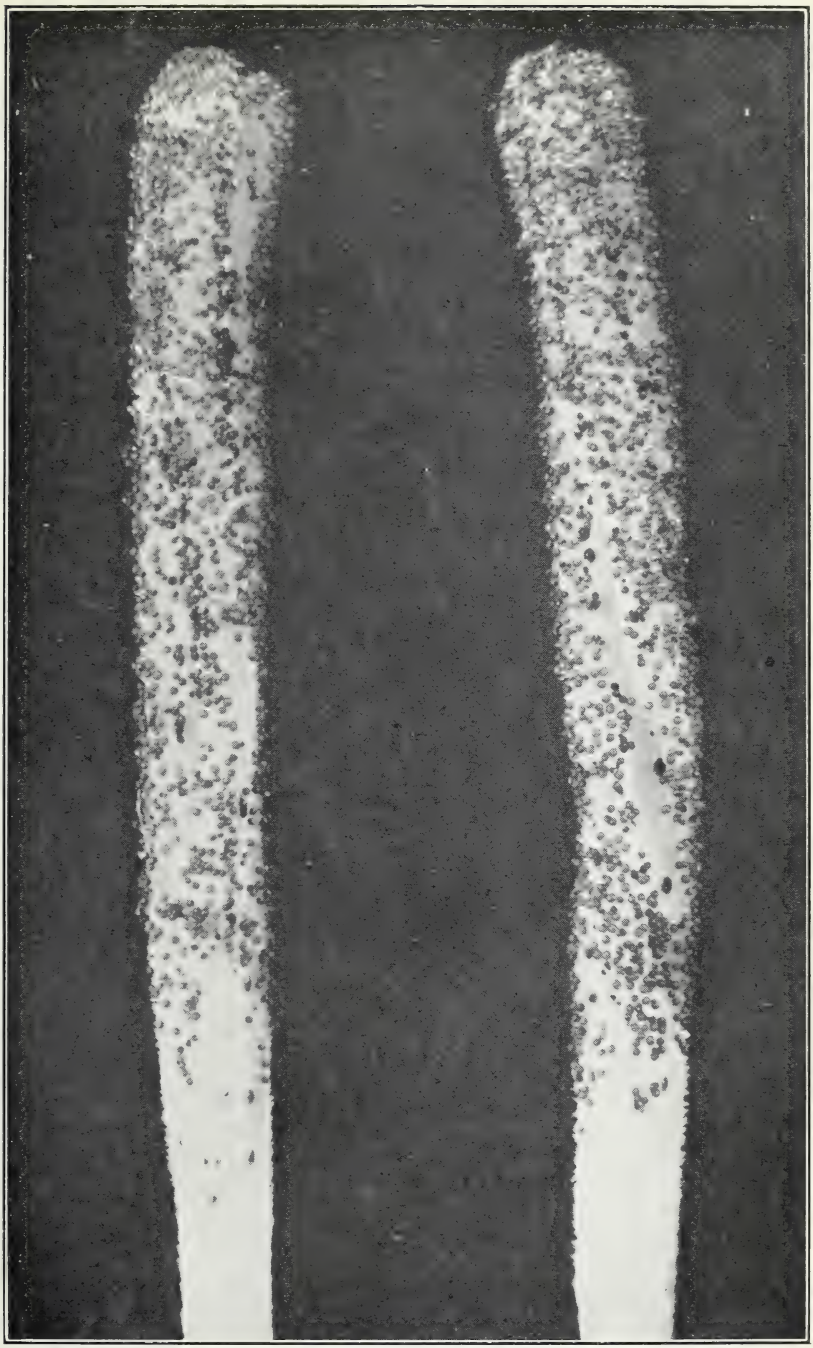
In order to attach the bag a slit is made in it nearly to the middle, so as to straddle the fruiting branch, and the bag is then tightly closed underneath the branch by means of a loop in a piece of insulated wire.

Photographed by W. F. Gilpin.

STIGMAS AND UPPER PORTION OF THE STYLE OF BAGGED FLOWERS OF PIMA COTTON.

These parts, dissected at about 3 p. m. of the day of anthesis from flowers which had been bagged to exclude insects, show the locus of automatic deposition of self pollen. The position of the lowest pollen grains indicates the height to which the sheath of the staminal column extended. The upper half of the stigmas is entirely free from pollen. (Magnified 10 times.)

Photographed by H. F. Loomis.



STIGMAS AND UPPER PORTION OF THE STYLE OF UNINCLOSED FLOWERS OF PIMA COTTON.

These parts were dissected at about 3 p. m. from uninclosed, naturally pollinated flowers. The whole stigmatic surface is covered with pollen grains. (Magnified 10 times.) Photographed by H. F. Loomis.

pollen-bearing flowers as early as 8 a. m., but it is believed that in every case the quantity applied was sufficient to have insured thorough pollination under normal conditions.

The resulting data, as given in Table 14, show that the stigmas had retained their receptivity in only a few of the flowers the pollination of which was deferred until the second day. Those which remained receptive, however, were as well fertilized as the flowers pollinated on the day of anthesis.[16]

TABLE 14.—*Results obtained by pollinating Pima cotton flowers on the day of anthesis and on the following day, showing the percentage of bolls matured and the mean number of seeds per boll.*

Time of pollination.	Flowers treated.	Percentage of bolls matured.	Mean number of seeds per boll.
1 p. m. of the day of anthesis	50	90.0±2.86	13.7±0.32
8 a. m. of the day following anthesis	48	8.3±2.70	[1] 13.2±3.05

[1] Omitting one of the four bolls matured which contained only a single seed, the mean number of seeds in the remaining bolls was 17.3±1.79.

LOCUS OF POLLEN DEPOSITION IN RELATION TO SELF-FERTILIZATION AND CROSS-FERTILIZATION.

Numerous examinations of flowers of Pima cotton when the corolla is still closed or is just beginning to expand have shown that if the flower is opened with sufficient care pollen is rarely found upon the stigmas at a height of more than 2 millimeters above the uppermost anthers, to which height the grains probably can be thrown by automatic discharge. A similar condition is found in flowers which have been bagged to prevent cross-pollination, even when the corolla has opened to a degree which in unbagged flowers would permit the ready access of pollen-carrying insects. As about 2.5 millimeters of the length of the stigmas is surrounded by the uppermost stamens, the portion upon which self pollen is automatically discharged therefore does not exceed, as a rule, 5 millimeters, or about half the average total length of the stigmas. The girdle of self-pollen grains deposited upon the stigmas at the height of the uppermost anthers, hence just above the summit of the staminal sheath, is shown in Plate VI. It has been pointed out that this zone, on which is lodged the great bulk of the automatically discharged self pollen, is so closely screened by the uppermost stamens with their short filaments as to be practically inaccessible to foreign pollen.

On the other hand, in flowers which open under natural conditions in a locality like Sacaton where pollinating insects are abundant, the entire surface of the stigmas usually becomes covered with pollen during the morning (Pl. VII). There can be little doubt that in this type of cotton, pollen which is found upon the upper half of the

[16] In Pima cotton the style normally drops off within 36 hours after the beginning of anthesis. A much longer duration of receptivity of the stigmas has been noted in other plants. Dorsey states (*14*, p. 116) that in the plum the "stigma remains receptive for a maximum period of about one week." It is reported by Anthony and Harlan (*5*, p. 528) that the stigmas of barley retained their receptivity during five days following emasculation, although the degree of fertilization effected diminished rapidly after the second day.

stigmas has been conveyed there by insects, whether it originated in the same or in other flowers, and that pollen which is lodged upon the basal quarter of the stigmas has been self-deposited.

The question suggests itself whether the rate of growth of the tubes from pollen grains deposited at different loci on the stigmas may be an important factor in determining the relative frequency of self-fertilization and of cross-fertilization. There is a considerable difference in the distance to the ovary to be traversed by the tubes from self-pollen grains automatically deposited near the base of the stigmas and by the tubes from insect-carried pollen grains deposited higher on the stigmas, the maximum difference, corresponding to the average length of the stigmas, being about 10 millimeters. This might be expected to give the self pollen a decided advantage in the time required for the pollen tubes to reach the ovary and to account, at least in part, for the observed preponderance of self-fertilization in Egyptian cotton.

Fertilization in Gossypium, according to Balls (*8*, p. 12), "is normally completed within 30 hours after the first opening of the flower, i. e., by the afternoon of the following day." An experiment was performed at Sacaton, Ariz., in 1921, in an endeavor to determine the length of time required to effect fertilization, or rather penetration of the ovary by the pollen tubes. Flower buds of Pima cotton were emasculated in the evening before anthesis and were pollinated at 1 p. m. the following day with pollen of the same variety. The pollen was deposited in some of the flowers at the apex and in others at the base of the stigmas. The pistils of approximately equal number of these flowers were then excised at the summit of the ovary at 8 p. m. of the day of pollination and at 5, 7, 9, and 11 a. m., and 1 p. m. of the following day.[a] A record was kept of the number of bolls which matured and of the number of seeds per boll, from which were computed the data given in Table 15.

TABLE 15.—*Degrees of fertilization attained in Pima cotton flowers pollinated at the apex and at the base of the stigmas, the pistils having been excised at various intervals following pollination.*

Pistil excision.		Apical pollination.			Basal pollination.		
Hour excised.	After pollination (hours.)	Flowers treated.	Percentage of bolls matured.	Mean number of seeds per boll.	Flowers treated.	Percentage of bolls matured.	Mean number of seeds per boll.
8 p. m.	7	45	0	.....	45	0	.....
5 a. m.	16	45	66.7±4.7	11.4±0.49	45	46.7±5.0	8.1±0.63
7 a. m.	18	45	77.8±4.2	14.7± .34	45	46.7±5.0	12.8± .45
9 a. m.	20	45	80.0±4.0	16.9± .23	45	68.9±4.7	12.7± .31
11 a. m.	22	45	82.1±3.9	16.5± .19	45	84.4±3.6	13.6± .28
1 p. m.	24	37	83.8±4.1	16.8± .18	40	87.6±3.5	15.1± .13

Fertilization did not occur in either the apically or the basally pollinated flowers of which the pistils were excised at 8 p. m. on the day of pollination. It is therefore evident that in this case more than

[a] Heribert-Nilsson (*22*) describes results obtained by this method of excising the style in computing the rate of pollen-tube development in Oenothera, which he found to average 4.5 millimeters per hour in mid-July. Fertilization did not occur in flowers of which the styles were excised earlier than 19 hours after pollination. This investigator also obtained evidence "that the pollen tubes of *O. gigas* grew slower in the styles of *O. lamarckiana* than *O. lamarckiana*'s own pollen tubes."

7 hours were required for penetration of the ovary by the pollen tubes.[a] Considering for the moment only the apically pollinated flowers, it is shown that at 5 a. m., or 16 hours after the pollen was deposited, the tubes had reached the ovaries of two-thirds of the flowers in number sufficient to fertilize on the average more than half of the mean number of ovules, which is 21. A slower rate of development of some of the tubes is indicated by the much more nearly complete fertilization of flowers in which the pistils were not excised until 9 a. m.

Some of the tubes doubtless had penetrated the ovary earlier than 5 a. m., but in estimating the mean rate of growth it may be assumed that the period of 16 hours represents the average length of time required. The further assumption is made, although proof is lacking, that germination began as soon as the pollen reached the stigmas. In the case of pollen applied at or near the apex of the stigmas, which average in Pima cotton one-third the length of the pistil exclusive of the ovary, it may be assumed that most of the grains germinated within 2 millimeters of the apex of the stigmas, or 28 millimeters above the base of the style, the average total length of stigmas and style being 30 millimeters. A growth of 28 millimeters in 16 hours indicates a mean rate of 1.75 millimeters per hour.[b] Self pollen automatically deposited at or near the base of the stigmas would be located on the average about 6 millimeters nearer the ovary, and tubes starting from this locus might be expected to penetrate the ovary 3½ hours in advance of the tubes from grains of foreign pollen starting near the apex of the stigmas. This would seem to give self pollen a decided advantage over foreign pollen, provided the conditions at both loci are equally favorable for the germination and development of the pollen.

Comparison of the rates of fertilization by apically and by basally deposited pollen, as stated in Table 15, indicates, however, that the base of the stigmas affords less favorable conditions for pollen development than the apex. For each interval after pollination, fertilization, as measured by the mean number of seeds per boll, was significantly less complete in the basally than in the apically pollinated flowers, and in the flowers excised at the latest hour, 1 p. m., the mean difference in favor of apical pollination amounted to 1.7 ± 0.22. The mean number of seeds per boll from basally pollinated flowers which had the pistils excised at 1 p. m. (24 hours after the pollen was deposited) was not significantly greater than the mean number from apically pollinated flowers which had the pistils excised at 7 a. m. (only 18 hours after the pollen was deposited).

Another experiment performed in 1921 yielded additional indications that pollen germinates and develops under relatively unfavorable conditions when deposited at the base of the stigmas. Pima

[a] In a similar experiment performed in 1920, however (see Table 24), a few bolls matured from Pima flowers of which the stigmas and style were excised 7½ hours after pollination, and these bolls contained relatively large numbers of seeds. Additional experiments performed in 1922, the complete data of which were not available in time for inclusion in this paper, gave convincing evidence that in Pima cotton within 8 hours after deposition of pollen on the stigmas the tubes can penetrate the ovary in number sufficient to fertilize more than half of the ovules.

[b] The fact that, in experiments performed in 1922, a few but comparatively well-fertilized bolls developed from apically pollinated flowers of Pima cotton of which the stigmas and style had been excised 8 hours after deposition of the pollen, indicates that in exceptional cases the average hourly growth rate may attain 3.5 millimeters.

flower buds were emasculated the evening before anthesis, other buds having been bagged at the same time to supply pollen. On the following morning approximately equal numbers of the emasculated flowers were pollinated (1) near the apex of the stigmas, (2) near the base, and (3) on the whole stigmatic surface. Record was kept of the number of bolls which matured from the several treatments and of the number of seeds in each boll, from which were computed the data given in Table 16.

TABLE 16.—*Degrees of fertilization in Pima cotton resulting from pollination of the apical and of the basal portion of the stigmas and of the whole stigmatic surface.*

Locus of pollination.	Flowers treated.	Percentage of bolls matured.	Mean number of seeds per boll.
Near the apex of the stigmas	94	80.8±2.73	14.7±0.28
Near the base of the stigmas	98	89.8±2.04	9.7± .29
Upon the whole length of the stigmas	100	98.0± .94	14.1± .22

A higher percentage of bolls matured from flowers pollinated near the base of the stigmas than from flowers pollinated near the apex, but the difference is not significant. On the other hand, the mean number of seeds per boll resulting from basal pollination was much smaller than that resulting from apical pollination, the difference having been 5.0±0.40 (more than 12 times its probable error). Pollination of the whole stigmatic surface yielded a significantly higher percentage of bolls matured than did either partial pollination, but did not show a significant difference in the mean number of seeds per boll as compared with pollination of the apical portion only. It may be inferred from this fact that a difference between the two halves of the stigmas is responsible for the inferior fertilization from basally deposited pollen, the extent of the area receiving pollen having been approximately the same in the apical and basal pollinations.

The data given in Tables 15 and 16 indicate [17] that when flowers of Pima cotton are emasculated and are pollinated artificially the basal region of the stigmas is a less favorable medium for the germination or development of pollen than is the apical region.[18] Care was taken in these experiments to apply as nearly as practicable equal quantities of pollen at both loci, but it was noted that the pollen adhered more closely to the stigmatic surface when apically deposited than when basally deposited. This was probably a factor in the superior fertilization from apical pollination. It is doubtful, however, whether this factor is operative in equal degree under natural conditions, for, with the stamens present, the close contact of the upper

[17] Additional and conclusive evidence that when emasculated flowers of Pima cotton are pollinated artificially better fertilization results with apical than with basal deposition of the pollen was obtained from two experiments in 1922, the complete data of which were not available in time to be included in this bulletin.

[18] Meade (*40*, p. 282) concluded from the results of his investigation of pollination in upland cottons, referred to under the heading "Structure of the flower in relation to pollination," that "most of the flowers with long stigmas projecting above the stamens do not become completely self-fertilized, as the anthers and stigmas are too widely separated." If limitation of pollen deposition to the basal region, as would be the case in flowers having long stigmas when pollinating insects are scarce, results generally in inferior fertilization, Meade's conclusion is probably well founded.

anthers with the base of the stigmas would favor the retention there of a greater number of pollen grains than in the case of emasculated flowers. Nevertheless, in experiments which afforded a comparison of the fertilization resulting from (*a*) automatic self-pollination in flowers that were not emasculated but in which the pollen was confined to the lower halves of the stigmas and (*b*) artificial pollination of emasculated flowers in which the bulk of the pollen was deposited on the upper halves of the stigmas, the latter treatment gave significantly better fertilization in six out of seven comparisons. On the other hand, data given in Table 16, which were fully confirmed by the results of an experiment performed in 1922, indicate that flowers receiving pollen on the upper halves of the stigmas only are fully as well fertilized as flowers receiving pollen on the whole stigmatic surface. It is probable, therefore, that apart from conditions affecting the adhesion of the pollen, there is a qualitative difference between different parts of the stigmas and that penetration of the tissues is effected more readily at the apex than at the base.

If in Pima cotton under natural conditions the pollen germinates and develops better at the apex of the stigmas than at the base, this probably more than offsets any advantage which the automatically deposited self pollen might derive from its nearness to the ovary. The structure of the flower in other types of cotton in which self-fertilization predominates increases the probability that the locus of pollen deposition is not a factor of much importance in determining the predominance of self-fertilization. In many varieties of upland cotton the uppermost stamens equal or even surpass the stigmas, so that the entire length of the latter is accessible to automatically discharged self pollen. The whole stigmatic surface is also accessible to insect-carried foreign pollen, no part of it being screened by a dense mass of stamens, as in the Egyptian cottons. Examination of open flowers of upland cotton when growing in close proximity to Pima shows the yellow pollen grains of the latter to be scattered over the whole surface of the stigmas, although usually most abundant near the apex.

RELATIVE EARLINESS OF ARRIVAL OF SELF-DEPOSITED AND OF INSECT-CARRIED POLLEN.

Especial interest in connection with the problem of the relative frequency of self-fertilization and of cross-fertilization attaches to the question whether there is an appreciable interval of time between the first arrival upon the stigmas of self pollen and of foreign pollen. There can be little doubt that if the stigmas greatly exceed the stamens, as is the case in Pima cotton, all pollen present upon the upper halves of the stigmas has been conveyed there by insects. When the pollen present on the stigmas is all of the same type it is impossible to determine what part of it has originated in the same flower and what part has been conveyed from other flowers by insects, but examination of flowers exposed to cross-pollination by a different type of cotton having readily distinguishable pollen showed that foreign pollen was being conveyed to the stigmas of most of the flowers. It may be assumed, therefore, that when pollen is present on the upper halves of the stigmas some of it has originated in other flowers.

OBSERVATIONS ON PIMA COTTON.

Examination in 1916 of numerous flowers, the corollas of which were open only 1 or 2 millimeters, showed none of them to have pollen present on the upper half of the stigmas. Flowers were examined in 1919 at intervals on July 27, beginning about two hours after sunrise, and on August 3, beginning about an hour after sunrise. Ten flowers were inspected at each interval on both dates. An endeavor was made to select in all cases flowers which had been fully exposed to the direct rays of the sun at and after sunrise and which should therefore have been favorably situated for the earliest possible opening of the corolla. Table 17 shows for each interval the percentage of the total number of flowers (10 in each case) in which an appreciable quantity of pollen was found upon the stigmas at a height sufficient to justify the conclusion that it must have been conveyed by insects.

TABLE 17.—*Rate of deposition of pollen on the upper portion of the stigmas in open-pollinated flowers of Pima cotton in 1919.*

Hour of observation.	Flowers having the upper portion of the stigmas pollinated (per cent).		Hour of observation.	Flowers having the upper portion of the stigmas pollinated (per cent).	
	July 27.	Aug. 3.		July 27.	Aug. 3.
6.30 a. m.	.....	0	10.30 a. m.	.....	50
7.30 a. m.	0	0	12.00 m.	.....	40
8.00 a. m.	10	.....	1.00 p. m.	.....	50
8.30 a. m.	50	20	2.00 p. m.	.....	70
9.30 a. m.	60	40	3.00 p. m.	.....	90
10.00 a. m.	70	.....			

On both dates, at the earliest hour of observation, the anthers of the flowers were discharging pollen, some of which presumably was reaching the lower half of the stigmas. Reference to Table 9 (p. 18) shows that at 8 a. m., the earliest hour when pollen was found in appreciable quantity upon the upper half of the stigmas, the average aperture of the corolla in Pima cotton is about 10 millimeters. A difference in the rapidity of pollination of the upper half of the stigmas on the two dates is indicated by the data in Table 17. Cross-pollination of 70 per cent of the flowers had taken place at 10 a. m. on July 27, and not until 2 p. m. on August 3. Yet the conditions would seem to have been more favorable to early pollination by insects on August 3, a clear sunny day, than on July 27 when the sky was overcast during the morning. The probable explanation is that on August 3 there was a marked scarcity of bees and other active pollen carriers in the cotton field.

Observations in 1920 indicated an earlier arrival of insect-carried pollen. On July 25, at 7.40 a. m., Pima flowers which were open about 10 millimeters were found to have numerous grains of pollen on the upper half of the stigmas, and in one flower, which had a corolla aperture of only 2.5 millimeters, much upland pollen was present. Most of the flowers examined on July 30, 1920, between 7.45 and 8 a. m. were already open from 5 to 20 millimeters and had numerous pollen grains on the upper half of the stigma, while in

the few flowers which were still closed at this time, self pollen was just beginning to be deposited in the interstamen region. Most of the flowers examined on August 8 at 7.30 a. m. were open from 5 to 10 millimeters and had pollen present on the upper half of the stigmas. Of 10 closed buds which were examined at the same hour, 7 had a very few grains of self pollen on the interstamen section of the stigmas. On August 21 the opening of the corolla had been retarded by the coolness of the early morning (minimum temperature 62° F.), but at 8.40 a. m., of 30 flowers which were open from 5 to 10 millimeters, only 3 or 4 had the upper half of the stigmas free from pollen. Many flowers which were open only about 5 millimeters had the stigmas well pollinated at this hour.

Observations in 1921 indicated that bees sometimes enter Pima flowers and deposit pollen upon the stigmas when the orifice of the corolla is still minute and that they occasionally do so by pushing aside the loosened petals before any orifice has formed. It was noted, however, on August 16 that most of the flowers had not been entered until the orifice had reached a diameter of 2 or 3 millimeters, in which stage most of the flowers had pollen present upon the upper half of the stigmas. The readiness with which insects enter unopen corollas seems to be controlled in some degree by the weather, for on the cloudy morning of August 18, when as late as 8 a. m. many of the buds showed no distinct orifice, although the petals were well loosened at the apex, most of the flowers in this stage of anthesis had more or less pollen upon the upper half of the stigmas.

It is evident that at the same locality there is considerable variation on different days in the earliness of the arrival of insect-conveyed pollen in the flowers of Pima cotton. This is doubtless to be accounted for by variations in the weather and in the number, kind, and habits of the pollinating insects. The conclusion seems warranted, however, that as a rule many of the flowers are entered by pollen-conveying insects soon after the expansion of the corolla has begun.

Evidence has already been presented that the automatic deposition of self pollen upon the stigmas does not commence much in advance of the time when the petals begin to unfold and that the quantity deposited before the corolla has developed an orifice is usually very small. It is probable that as a rule the interval of time between the first arrival of self pollen and of foreign pollen does not exceed half an hour and that frequently foreign pollen begins to arrive before any considerable quantity of self pollen has been deposited upon the interstamen region of the stigmas.

OBSERVATIONS ON UPLAND COTTON.

It has been pointed out that when the two types are growing under similar conditions the corollas of upland varieties open somewhat more rapidly than those of Pima cotton. On the other hand, while in Pima the opening of the anthers always precedes that of the corolla, the anthers of upland varieties frequently do not begin to open until the expansion of the corolla has begun, while in some upland varieties the anthers are often still closed when the corolla is partly open. In the Cleveland and Dixie Triumph varieties on July 27, 1920, the anthers were observed to be still closed in flowers which had opened sufficiently to allow the stigmas to become well covered with foreign

pollen. In most of the 20 upland varieties upon which observations were made in 1920, it appeared, however, that the first arrival upon the pistil of self pollen and of foreign pollen was virtually simultaneous.

In 1921 several instances were recorded of the occurrence of foreign pollen upon the stigmas of partly open upland flowers in which self-pollination had not yet taken place, the anthers being still closed. The presence of foreign pollen upon the stigmas was readily determined in these cases, as the upland varieties in 1920 and 1921 were grown in close proximity to Pima cotton, the bright-yellow pollen grains of which are easily distinguished from the whitish grains of the upland pollen. It may be concluded, therefore, that in upland cottons the interval between the beginning of automatic self-pollination and that of pollination by insects is at most a very brief one and that not infrequently foreign pollen reaches the stigmas in advance of self pollen.

DEPOSITION OF SELF POLLEN AND OF FOREIGN POLLEN BY INSECTS.

Evidence of the degree in which cross-pollination occurs in the two types of cotton was afforded in 1920 by examination of flowers borne by isolated plants of Pima (Egyptian) cotton distributed through a plat of Acala (upland) and of flowers borne by isolated Acala plants distributed through a plat of Pima in an experiment described on a preceding page, the object of which was to determine the percentages of natural hybrids or vicinists which would be produced under these conditions (Table 5). The isolated plants were separated from each other by several plants of the other variety, and only one flower was allowed to open daily on each isolated plant. It is therefore certain that most, if not all, of the pollen from other flowers which reached the stigmas of these plants was of the other type; hence, readily distinguishable from the self pollen. Observations on several days (July 30 to August 3) during a period when both types were producing flowers in approximately equal numbers gave the results stated in Table 18.

TABLE 18.—*Relative proportions of self pollen and of foreign pollen present on the upper portion of the stigmas of Pima and of Acala plants isolated among plants of the other type.*

[The figures indicate the number of flowers belonging to each category.]

Nature of the pollen present.	On the stigmas of—	
	Pima (Egyptian).	Acala (upland).
Self pollen only	6	1
Self pollen predominating	19	11
Approximately half-and-half	5	9
Foreign pollen predominating	4	4
Foreign pollen only	0	0
Total flowers examined	34	25

Since in Pima cotton the pollination of the upper portion of the stigmas is effected by insects, the data in Table 18 point strongly

to the conclusion that much of the pollen conveyed to the stigmas by this agency originates in the same flower and that the preponderance of self-pollination is an important factor in the preponderance of self-fertilization in cotton. It would appear also that under like conditions as to climate and insect fauna upland cotton is more subject than Egyptian to cross-pollination, self pollen having predominated in 73 per cent of the Egyptian flowers and in only 48 per cent of the upland flowers. The latter conclusion is supported by the results of an examination at Sacaton, Ariz., in 1920, of flowers of Pima and of upland cotton which were growing in adjacent rows. There was much more Pima pollen on the stigmas of the upland flowers than of upland pollen on the Pima stigmas.

Further observations were made in 1921. The flowers examined were taken from adjacent rows of Pima and of upland, in every case from the side of the plant which faced a plant of the other type. They were collected in pairs, one flower of each type from opposite or nearly opposite plants. Pima flowers were preferred which were borne at approximately the same height above the ground as the upland flowers. The Pima plants were flowering somewhat more freely than the upland, but the difference in this respect was not great. The results are stated in Table 19.

TABLE 19.—*Flowers of Pima and of upland cottons having different quantities of pollen of the other type present on the stigmas.*

[The figures indicate the number of flowers belonging to each category.]

Type of cotton.	Flowers examined.	Pollen of other type on stigmas.			
		None.	Fewer than 10 grains.	Ten or more grains, but less than half of total pollen present.	Half or more than half of total pollen present.
Pima (Egyptian)	100	31	57	11	1
Upland	100	10	47	43	0

Of the total number of Pima flowers examined 31 per cent had no upland pollen present on the stigmas and 88 per cent had fewer than 10 grains of upland pollen. Of the total number of upland flowers only 10 per cent had no Pima pollen on the stigmas and only 57 per cent had fewer than 10 grains of Pima pollen. These percentages do not, however, fully indicate the difference between the two types, for of the flowers which were classed as having fewer than 10 grains of pollen of the other type on the stigmas, the number which had received very few grains (1 to 3) was much greater in the case of Pima than in the case of upland. There can be no doubt, therefore, that when both types of cotton are growing side by side and are flowering at approximately the same rate Pima pollen is conveyed to the upland stigmas in greater quantity than upland pollen to the Pima stigmas. This must be a very important factor in the observed greater prevalence of cross-fertilization in upland than in Pima cotton.

An answer to the question why Egyptian pollen is conveyed to the stigmas of upland cottons in greater quantity than upland pollen to the Egyptian stigmas is to be sought in a consideration of the habits of the pollen-carrying insects in relation to the ontogeny of the flower. At Sacaton, Ariz., the honeybee and at times wild bees of the genus Melissodes are the insects which enter most frequently the corollas of Pima cotton. It has been observed that many of the flowers are first entered when the petals are just beginning to unfold, occasionally even before there is an actual aperture at the apex of the corolla, under which conditions the bees almost invariably come into contact with the reproductive organs, taking up and depositing pollen. When the Pima corollas are in this early stage of expansion the anthers of upland cotton frequently are still closed or are just beginning to split, so that little, if any, pollen of this type is available for transfer to the Pima stigmas. On the other hand, many of the upland corollas at this time are quite as accessible as those of Pima to entry by the insects, and this accounts for the frequent deposition of Pima pollen upon the upland stigmas before any self pollen has reached the latter.

By the time the upland anthers have begun to discharge pollen freely most of the Pima corollas have opened to a degree which allows honeybees to reach the nectaries by crawling down the inside of the petals without touching the reproductive organs and to make their exit in the same manner. It is a relatively infrequent occurrence for the honeybees to touch stigmas or stamens in entering or leaving a well-opened Pima flower. On the other hand, the wild bees (*Melissodes* spp.) apparently do so regularly. It would be interesting to ascertain whether foreign pollen is conveyed in greater quantity to the Pima flowers during periods when Melissodes are visiting them in large numbers than when honeybees are the predominant visitors.

POLLEN-CARRYING INSECTS AT SACATON.

There is little doubt that natural cross-pollination in cotton is effected almost solely by the agency of insects. The nature of the pollen grains of Gossypium is unfavorable to their transportation by currents of air. Allard (*2*, p. 256), however, found that glass plates smeared with vaseline and exposed in cotton fields in northern Georgia collected considerable quantities of cotton pollen. On the other hand, Balls (*3*, p. 117), using the same method for the detection of wind-disseminated pollen in Egypt, obtained negative results.

No systematic study of the insects which visit cotton flowers has been attempted in connection with these investigations, but numerous specimens have been collected at Sacaton, Ariz., and notes have been made upon the efficiency as pollinators of those which most frequently enter the flowers. The writer is indebted for the identification of the specimens to Dr. L. O. Howard, Chief of the Bureau of Entomology, United States Department of Agriculture.[19]

[19] The several groups were identified by the following specialists: Hymenoptera, by S. A. Rohwer; Coleoptera, by E. A. Schwarz; Hemiptera, by Miss E. A. Wells and W. L. McAtee; Orthoptera, by A. N. Caudell; Lepidoptera, by H. C. Dyar; and Diptera, by J. M. Aldrich.

Various Hymenoptera are the most efficient carriers of cotton pollen at Sacaton, Ariz.,[20] as is probably the case wherever cotton is grown. The honeybee and wild bees (*Melissodes* spp.) are the most important cotton pollinators in this locality.

The honeybee (*Apis mellifica* L.) is very assiduous in its visits to cotton flowers, although sometimes preferring the extrafloral nectaries to those within the flower.[21] Nevertheless, this insect probably holds first rank at Sacaton, Ariz., as a conveyor of cotton pollen, especially among Pima flowers. As was noted on a preceding page, honeybees entering and emerging from the flowers when the petals are just beginning to unfold almost invariably come in contact with the reproductive organs. Later in the morning, when a sufficient aperture has developed, the bees usually crawl down the inside of the petals without touching the stigmas or stamens and make their exit in the same manner.[22] Occasionally, however, the honeybee touches the staminal column and stigmas even when the corolla is fully open, this being especially likely to happen when the insect is confused by the entrance of another individual.

At times wild bees of the genus Melissodes (*M. agilis agilis* Cress., *M. agilis aurigenia* Cress., *M. tristis* Ckll.) are even more efficient pollinators than honeybees. It was observed at Sacaton, Ariz., in 1921 that Melissodes were much more numerous in the cotton fields toward the close of the season than was the case earlier in the summer and that, unlike the honeybee they commonly crawl over the stigmas and staminal column of open flowers in order to reach the nectaries at the base of the corolla. Another bee, *Megachile parallela* Smith, is remarkable for the quantity of pollen it carries but is apparently a much less frequent visitor.

Large wasps of the genus Campsomeris, especially *C. dives* Prov., also frequent the cotton flowers. They apparently prefer upland varieties, which have a shallow, widely flaring corolla, to the Egyptian cotton with its deep and relatively narrow corolla. These insects carry much pollen from flower to flower. Their habits of grasping the stamens with their legs when entering and leaving the flower and of pressing the stamens against the stigmas while drinking from the intrafloral nectaries doubtless also contribute materially to self-pollination.

Other Hymenoptera which have been observed to carry cotton pollen at Sacaton, Ariz., are *Cerceris* sp., *Dasymutilla ursula* Cress., and the carpenter bees (*Xylocopa arizonensis* Cress. and *X. varipuncta* Patt.)[23] A species of Pepsis seems to be more efficient in distributing pollen within the flower than in transferring pollen from one flower to another.

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[20] Allard states (*2*, p. 254) that in northern Georgia *Melissodes bimaculata* Le P. and the honeybee are "the most abundant and constant visitors of cotton." Allard gives a list of insects observed to visit cotton flowers in that region, with interesting notes on the itineraries of honeybees and bumblebees among the cotton plants. In a later paper by the same author it is stated (*3*, p. 680) that of 129 observed entrances of insects into cotton flowers in the Georgia locality, 45 were by species of Melissodes and 45 by honeybees. It was noted that the wild bees were much the most frequent visitors when the observations began, while later the honeybees increased the frequency of their visits.

[21] Seasonal variations in the habit of the honeybee in this respect were noted by Allard in Georgia (*2*, pp. 256, 257).

[22] The same habit was observed at Palestine, Tex., by Shoemaker (*43*).

[23] According to Shoemaker (*43*) bumblebees were the most active pollinators at Palestine, Tex., in September, but seemed to be more efficient in insuring thorough self-pollination than in effecting cross-pollination.

Species of Coleoptera which have been observed to visit cotton flowers and to carry more or less pollen on their bodies, but which are probably of minor importance as pollinators, are *Megilla maculata fuscilabris* Mulsant, *Diabrotica 12-punctata* Fabr., *D. balteata* Leconte, *D. trivittata* Mannerheim, *Collops vittatus* Say, *Phalacrus penicellatus* Say, and the fruit beetle (*Allorhina nitida* L.). The same remarks apply also to certain Hemiptera (*Zelus renardi* Kol., *Congus* sp., *Corizus hyalinus* Uhler, and *Apiomerus spissipes* Say) and to *Nemotelus trinotatus* Mel., of the order Diptera.

A small and very slender black beetle *Conotelus stenoides* Murray, which is extremely abundant at Sacaton, Ariz., sometimes effects its entrance to the flower as a result of an abnormal separation of the bases of the petals while the tip of the bud is tightly closed and occasionally makes its way into flowers which have been bagged to prevent cross-pollination. The small size and the smoothness of the body of this insect make it unlikely that it has any importance as a carrier of pollen from flower to flower. Another small beetle occasionally found in bagged flowers is *Notoxus calcaratus* Horn, and one of these insects having a single grain of pollen attached to its head was found in a flower thus inclosed. It is unlikely, however, that an appreciable quantity of pollen is transferred from flower to flower under these circumstances.[24]

The method of inclosing the flower in a paper bag, illustrated in Plate V, has proved very efficacious as a means of preventing cross-pollination. Many thousands of flowers have been "selfed" in this manner at Sacaton during the past eight years and none of the resulting progenies have given clear evidence of contamination from the access of foreign pollen. The efficacy of this method of excluding pollen transfer was tested by an experiment performed in 1915, in which 40 flower buds of Pima cotton were carefully emasculated and bagged in the ordinary manner the evening before the corolla was due to open. No artificial pollination was done, and none of the flowers developed a boll. The experiment was repeated in 1920, using 100 flowers, not one of which developed a boll.

RELATIVE COMPATIBILITY OF LIKE AND OF UNLIKE POLLEN.

The possibility suggests itself that pollen of another variety may be less compatible than self pollen or pollen of the same variety and that this, in addition to the preponderance of self-pollination, may be a factor in the greater prevalence of self-fertilization. To test this possibility, comparison was made of the degrees of fertilization attained when pollens of different degrees of relationship were applied separately to the Pima stigmas.

COMPARISON OF SELF-POLLINATION AND OF CROSS-POLLINATION WITHIN THE VARIETY.

An experiment was performed in 1921 in which some flowers were self-pollinated and others cross-pollinated on the same plants. Two Pima populations, the continuously open-pollinated stock and a

[24] Robson (*41*) observed in the West Indies that thrips enter the corolla before it has developed an orifice and concluded that cross-pollination may be effected by the agency of this insect.

family which has been strictly inbred (selfed) during seven generations, were used in this experiment. The treatments were as follows:

(A) Flower emasculated and bagged the evening before anthesis and pollinated the following morning with pollen from another flower on the same plant.

(B) Treatment similar to the above except that pollen from other plants of the same variety was used, these having been of the open-pollinated stock, not of the inbred family.

The use in treatment A of pollen from another flower on the same plant insured self-fertilization unless somatic variation had occurred, and of this there was no indication.[25] The possibility that the pollination might have been less thorough in the self-pollinated than in the cross-pollinated flowers also was eliminated by this method. In addition to the percentages of bolls matured and the mean numbers of seeds per boll, determinations were made of the mean weights and the germination percentages of the seeds resulting from the two treatments. The data of this experiment are given in Table 20.

TABLE 20.—*Comparison of the results of self-pollination and of cross-pollination within the variety in an open-pollinated stock of Pima cotton and in a family which had been closely inbred during seven generations.*

[All flowers emasculated.]

Population.	Pollination.	Flowers treated.	Percentage of bolls matured.	Mean number of seeds per boll.	Mean weight of 100 seeds.	Percentage of germination.
Open-pollinated	Self	165	91.0±1.5	17.0±0.17	12.8±0.04	90.8±0.9
Do	Cross	162	85.8±1.8	16.7± .18	12.6± .08	88.2±1.0
Difference			5.2±2.3	.3± .25	.2± .09	2.6±1.3
Inbred	Self	155	92.9±1.3	17.2± .16	13.5± .05	86.8±1.0
Do	Cross	151	86.3±1.7	17.5± .18	12.6± .08	89.7± .9
Difference			6.6±2.1	.3± .24	.9± .09	2.9±1.3

The data given in Table 20 show little difference in the results of the two treatments in either population. The only differences that appear to be significant occurred in the inbred population in respect to the percentage of bolls matured and the mean weight of seeds, self-pollination having given the higher value in both cases. In the mean number of seeds, the real criterion of the relative completeness of fertilization, neither population showed a significant difference. The outcome of this experiment warrants the conclusion that within the Pima variety there is practically no difference in compatibility between self pollen and pollen from other plants.

[25] Emoto (*16*) tested species of Primula, Brassica, Hyacinthus, Freesia, etc., as to the comparative effects of autogamy (fertilization by pollen of the same flower), geitonogamy (fertilization by pollen from another flower of the same plant), and xenogamy (fertilization by pollen from another plant). The criteria used were fruitfulness, length, and width of the capsules, number of seeds per capsule, weight of seeds, and germination of seeds. This author concluded that geitonogamy was superior to autogamy in very few cases. Darwin (*13*, p. 329) concluded from the results of his experiments with plants belonging to numerous families that "in very few cases did crossing different flowers of the same plant as compared with selfing a flower with its own pollen appreciably increase the number of seeds produced."

COMPARISON OF CROSS-POLLINATION WITH RELATED AND WITH UNRELATED POLLEN.

Flower buds on several Pima cotton plants in 1914 were emasculated and bagged in the evening and were pollinated the following morning, some with pollen from other plants of the same variety and others with pollen of the Gila variety of Egyptian cotton. The results, which show no significant difference in fertilization from the two pollinations, are given in Table 21.

TABLE 21.—*Fertilization of emasculated Pima flowers resulting from cross-pollination with pollen of the same variety and with pollen of the Gila variety of Egyptian cotton.*

Cross-pollination with—	Number of bolls matured.	Mean number of seeds per boll.
Pima (Egyptian) pollen	69	15.8±0.48
Gila (Egyptian) pollen	157	15.6± .36
Difference		.2± .60

A similar experiment was performed in 1917 on plants of a Pima family which had been strictly inbred during three successive generations. The cross-pollinations of emasculated flowers were made with pollen from (1) sister plants of the same inbred family, (2) plants of the continuously open-pollinated stock of the Pima variety, and (3) plants of the Gila variety. Table 22 gives the results of this experiment, which show that while a greater number of seeds per boll resulted from the application of pollen of another variety of the Egyptian type as compared with that from pollen of related and of unrelated plants of the same variety, the differences were not significant. It may be concluded, therefore, that within the limits of the Egyptian type there is no important difference in the compatibility of pollen derived from sister plants of a presumably homozygous strain, from unrelated plants of the same variety, and from another variety.

TABLE 22.—*Fertilization of emasculated Pima flowers resulting from cross-pollination with pollen from sister plants and from unrelated plants of the same variety and with pollen of the Gila variety.*

Cross-pollination with pollen from—	Flowers treated.	Percentage of bolls matured.	Mean number of seeds per boll.
Sister plants of the Pima variety	61	98±1.2	15.9±0.29
Unrelated plants of the Pima variety	90	99± .7	16.3± .19
Plants of the Gila (Egyptian) variety	31	97±2.1	17.1± .36

Experiments will be described next in which cross-pollination within the Pima variety was compared with cross-pollination with a wholly different type, the Acala variety of upland cotton. The results of such an experiment, performed in 1920, are presented in Table 23 (upper part) and indicate that the mean number of seeds per boll and the germination percentage of the seeds were significantly higher from flowers which had received pollen of the other

type, the differences having been, respectively, six and four times the probable error. This outcome being somewhat unexpected the experiment was repeated in 1921. Five plants of an inbred (seven generations selfed) Pima family were used as mothers. Flowers were emasculated before anthesis and were cross-pollinated the following day, some with pollen from plants of the bulk Pima stock, others with pollen of Acala (upland). The flowers which received both pollinations were borne on the same plants. The results of this experiment are also presented in Table 23 (lower part).

TABLE 23.—*Fertilization of emasculated Pima flowers, some of which were cross-pollinated with pollen of the same variety and others with pollen of the Acala variety of upland cotton in 1920 and 1921.*

Season and character of pollination.	Flowers treated.	Percentage of bolls matured.	Mean number of seeds per boll.	Mean weight of 100 seeds (grams).	Percentage of germination.
Season of 1920:					
Pima pollen	45	80.0±4.0	12.9±0.45	12.5±0.26	92.5±0.9
Acala (upland) pollen	48	79.2±3.9	16.2± .29	12.6± .08	96.6± .5
Difference		.8±5.6	3.3± .53	.1± .27	4.1±1.0
Season of 1921:					
Pima pollen	175	86.3±1.7	17.5± .18	12.6± .08	89.7± .9
Acala (upland) pollen	176	93.1±1.3	17.6± .16	13.0± .10	93.0± .8
Difference		6.8±2.1	.1± .24	.4± .13	3.3±1.2

In 1921 application of pollen of a different type, Acala, resulted in a slight but possibly significant increase in the percentage of bolls matured, but did not effect more nearly complete fertilization, the mean number of seeds per boll having been practically the same as that obtained by cross-pollination within the variety. There were indications that fertilization by the more foreign pollen slightly increased the weight and percentage germination of the seeds, although the differences were scarcely significant.[a]

Considering the whole series of experiments in which pollens of different degrees of foreignness were compared as to their relative efficiency in fertilizing Pima flowers, it may be concluded that fertilization by the more foreign pollen is consistently neither better nor poorer than that effected by the more nearly related pollen. The conclusion holds good whether comparison is made (1) between pollen of the same plant or of a sister plant of an inbred family and pollen of unrelated plants of the same variety, or (2) between pollen of the same variety and pollen of another variety of the same type of cotton (Gila, Egyptian). On the other hand, comparison

[a] Two experiments performed in 1922, the detailed results of which were not available in time to be included in this bulletin, showed that bolls from Pima flowers pollinated with upland pollen (Lone Star variety) as compared with bolls from Pima flowers pollinated with Pima pollen, contained significantly greater mean numbers of seeds, the increases from extra-varietal pollination, in the two experiments, respectively, having been 9 and 15 per cent and having been 4.5 and 4.4 times the probable error of the difference. The reciprocal pollinations on Lone Star (upland), on the contrary, gave in both experiments a greater mean number of seeds per boll from the flowers pollinated with Lone Star pollen than from the flowers pollinated with Pima pollen, the decreases from extra-varietal pollination of Lone Star in the two experiments having been, respectively, 19.3 and 5.4 per cent, although the decrease was barely significant in the first experiment (difference 3.2 times its probable error) and was not significant in the second experiment. These results might be taken as indicating superior vigor of the Lone Star pollen but, when the two pollens were tested in sugar solution, the Pima pollen ejected somewhat more rapidly and completely than the Lone Star pollen.

of the fertilization of Pima flowers by Pima pollen and by pollen of a very different but still compatible type of cotton (upland), shows that in several cases somewhat better fertilization resulted from the foreign pollen. This is of especial interest in view of the fact that the pistils of the upland varieties used in these experiments (Acala and Lone Star) are much shorter than the Pima pistils.

RAPIDITY OF GERMINATION AND DEVELOPMENT OF DIFFERENT POLLENS.

Comparison of like and unlike pollens in respect to the rapidity of development of the tubes was the object of an experiment performed in 1920. Pima flower buds were emasculated in the evening and were thoroughly cross-pollinated the following day, some with pollen from other Pima plants, others with pollen of Acala (upland). The pistils were then excised at the summit of the ovary at successive intervals of time.

TABLE 24.—*Comparison of the rapidity of germination and development of different pollens, as shown by Pima flowers with pistils excised at successive intervals of time after pollination.*

Pistil excision.		Pollination with Pima (Egyptian) pollen.			Pollination with Acala (upland) pollen.		
Hour excised.	After pollination (hours).	Flowers treated.	Percentage of bolls matured.	Mean number of seeds per boll.	Flowers treated.	Percentage of bolls matured.	Mean number of seeds per boll.
8 p. m.	7½	40	7.5±2.8	13.7±1.4	37	0	0
5 a. m.	16½	39	61.5±5.2	11.5± .6	35	45.7±5.7	13.7±0.8
7 a. m.	18½	35	63.0±5.5	13.5± .6	35	51.5±5.7	14.5±1.0
9 a. m.	20½	36	91.8±3.0	15.7± .6	34	73.5±5.1	15.6± .6
11 a. m.	22½	35	80.0±4.6	15.6± .5	39	95.0±2.4	15.0± .4
1 p. m.	24½	26	92.3±3.5	14.4± .5	25	84.0±4.9	14.5± .7

The data of this experiment, as given in Table 24, indicate that the Pima and the upland pollen were equally efficient in fertilizing the Pima flowers. There was also no important difference between the two pollens in the rate of growth of the tubes, as indicated by the degrees of fertilization at successive intervals after pollination, except that of the flowers excised 7½ hours after pollination, three which had been pollinated with Pima pollen set bolls, while no bolls developed from flowers pollinated with Acala pollen which had been excised at this early hour.

POLLEN COMPETITION AS A FACTOR IN SELF-FERTILIZATION AND CROSS-FERTILIZATION.

Evidence was given on a preceding page that when the several pollens were applied separately to the Pima stigmas, approximately equal compatibility of self pollen, pollen from other plants of the same variety, pollen of another Egyptian variety, and pollen of another type of cotton (upland) was shown by the degree of fertilization effected. Fairly satisfactory evidence also was presented that the tubes of Pima and of upland pollen grow with approximately equal rapidity when these pollens are applied separately. It remains to consider whether, when different pollens are in competition on the stigmas of the same flower, selective fertilization occurs.

RESULTS OF OTHER INVESTIGATORS.

Balls (*7*, pp. 222, 223; *8*, pp. 122–125), using "a method of mixed pollination, whereby the stigma of a flower received equal quantities of (1) self pollen from its own anthers and (2) pollen from another plant," found that the seed produced by Egyptian flowers receiving both self pollen and upland pollen yielded 10 hybrids out of 330 plants, or somewhat less than 3 per cent. The percentage was about the same with the reciprocal cross-pollination upland × self + Egyptian. On the other hand, when stigmas of Egyptian or of upland cotton were pollinated simultaneously with approximately equal quantities of self pollen and of pollen from Egyptian × upland F_1 plants, the resulting percentages of hybrids were 20 and 28, respectively. These results seem to indicate that pollen of the conjugate generation of a hybrid between very different types of cotton, when applied to the stigmas of one of the parent types, is better able to compete with the self pollen than is the pollen of the other parental type. Balls does not describe in detail the method used in this experiment; but if automatic self-pollination of the base of the stigmas was not prevented, this would account in part for the very low percentages of hybrids when pollen of the other type, in addition to self pollen, was applied.

Longfield Smith (*44*) performed experiments the object of which was to produce the largest possible number of F_1 hybrids with the least expenditure of labor. To this end the stigmas of unemasculated flowers of sea-island cotton and of cotton of an upland type, said to be native in St. Croix, were smeared at 7.30 to 8 a. m. with pollen of the other species. Sea island × St. Croix yielded from 30 to 40 per cent of hybrids, while the reciprocal cross-pollination yielded 70 per cent. The much greater percentage of hybrids obtained from the reciprocal was attributed by this experimenter to the earlier opening of the anthers in the sea-island than in the St. Croix flowers, which allowed automatic self-pollination to begin earlier in the former than in the latter. The very high percentages of hybrids obtained from unemasculated flowers of the St. Croix cotton in this experiment seem to indicate a decided "prepotency" of the foreign pollen.

The hitherto unpublished data of an experiment conducted by Argyle McLachlan, under the direction of O. F. Cook, at Yuma, Ariz., in 1910 and 1911 are also of interest in this connection. Flowers of Egyptian cotton (Yuma variety) were pollinated with pollen of the same variety and with upland pollen, and flowers of upland cotton (Triumph and Durango varieties) were pollinated with pollen of the same variety and with Egyptian pollen. The flowers to be pollinated were emasculated before their anthers had opened and were then inclosed in bags. The flowers which were to furnish the pollen were bagged before their corollas had opened. Pollination was done as soon as the anthers opened.[26] In some cases the two kinds of pollen were applied simultaneously, in other cases the second kind was applied after intervals of 15, 30, and 60 minutes, using

[26] The original records of this experiment apparently have been lost, but the results are stated in a memorandum prepared by Mr. McLachlan on August 9, 1911, from which Table 25 has been compiled. Further details in regard to the procedure followed were supplied from memory by Mr. McLachlan in a letter to the writer dated December 5, 1919.

for the first pollination in some cases pollen of the same variety, in other cases pollen of the other type. Progenies from the resulting bolls were grown in 1911. The percentages of hybrids obtained from the various pollinations are shown in Table 25.

TABLE 25.—*Hybrids resulting in 1911 from double pollinations of Egyptian and upland cotton flowers at Yuma, Ariz., in 1910.*

Method of pollination.	Egyptian as the female parent.		Upland as the female parent.	
	Plants grown.	Percentage of hybrids.	Plants grown.	Percentage of hybrids.
Both pollens applied simultaneously	164	18 ±2.0	158	32 ±2.5
Egyptian pollen applied first, upland pollen 15 minutes later	184	8 ±1.3	142	56 ±2.8
Egyptian pollen applied first, upland pollen 30 minutes later	146	14 ±1.9	160	24 ±2.3
Egyptian pollen applied first, upland pollen 60 minutes later	151	13 ±1.8	121	59 ±3.0
Average for the Egyptian followed by upland pollination	.....	11.7±1.0	.....	46.3±1.6
Upland pollen applied first, Egyptian pollen 15 minutes later	118	5 ±1.4	107	51 ±3.3
Upland pollen applied first, Egyptian pollen 30 minutes later	123	10 ±1.8	93	36 ±3.4
Upland pollen applied first, Egyptian pollen 60 minutes later	166	7 ±1.3	107	47 ±3.3
Average for the upland followed by Egyptian pollination	.....	7.3± .9	.....	44.7±1.9
Totals for the two types	1,052	10.8± .6	888	42.3±1.1

The data given in Table 25 show no consistent differences in the percentages of hybrids depending upon whether pollen of the same or of the other variety was applied first or upon the length of the interval between the application of one and the other pollen. When Egyptian cotton was used as the female parent, deferring the application of the upland pollen until one hour after the Egyptian pollen was applied resulted in an apparent reduction in the percentage of hybrids as compared with that resulting from simultaneous application of the two pollens, but the difference was not significant. On the other hand, deferring the application of the Egyptian pollen until an hour after the upland pollen was applied, so far from increasing the percentage of hybrids, resulted in an apparently significant decrease as compared with the percentage from simultaneous application. With upland cotton as the female parent the percentage of hybrids when application of the Egyptian pollen was deferred one hour was significantly greater than in the case of simultaneous application of the two pollens, but a much greater and more significant increase in the percentage of hybrids resulted from deferring application of the upland pollen until one hour after the Egyptian pollen was applied. These results are inconsistent and seem inexplicable, but at any rate they increase the probability that the slight differences, under natural conditions, in the time of the arrival of self pollen and of foreign pollen, as noted on a preceding page, are of little consequence in determining the relative degree of self-fertilization and of cross-fertilization.

The total number (1,052) of Egyptian flowers which were double pollinated in the McLachlan experiment yielded 10.8±0.6 per cent of hybrids, and the total number (888) of upland flowers yielded 42.3±1.1 per cent of hybrids, the latter figure representing only a

small departure from the 50 per cent to be expected if the two pollens compete upon equal terms. It would therefore seem that while Egyptian pollen is very strongly prepotent over upland pollen on the Egyptian stigmas, there is no corresponding prepotency of upland pollen on the upland stigmas.

EXPERIMENTS AT SACATON, ARIZ.

DEPOSITION OF THE TWO POLLENS NOT SIMULTANEOUS.

An experiment was performed in 1916 with the object of determining in what degree pollen of a distinct but related variety may compete with automatically deposited self pollen. Flower buds on several plants of Pima cotton were bagged early in the morning but were not emasculated. At about 10.30 o'clock the stigmas were thoroughly smeared with pollen of the Gila variety, which is also of the Egyptian type, and the bags were replaced. Seed from the resulting bolls was planted in 1917 and the plants were thinned in such manner as to avoid any selection. Of the 240 plants which remained after thinning 34, or 14.2±1.5 per cent, were classed as hybrids (Pima × Gila F_1) and the remainder as pure Pima. We may therefore conclude that 86 per cent of the ovules had been self-fertilized in flowers which had received an abundance of foreign pollen.

In order to ascertain whether pollen of a very different type may compete better with self pollen than pollen of a related variety, an experiment was performed in 1919. Two flowers each on a number of plants of Pima (Egyptian) cotton were bagged in the evening, but were not emasculated, thus permitting automatic self-pollination to proceed in the normal manner. At about noon of the following day the stigmas of one flower on each plant were smeared with pollen of the Gila variety of Egyptian cotton, and the stigmas of the other flower were smeared with pollen of the Acala variety of upland cotton. In 1920, populations were grown from the seed resulting from each self + cross-pollination, all plants which developed having been left in place until the percentages of first-generation hybrids (Pima × Gila and Pima × Acala, respectively) had been determined. The results are stated in Table 26 (upper part).

TABLE 26.—*Hybrids resulting from seed produced by flowers of Pima cotton which, in addition to having been automatically self-pollinated, had been cross-pollinated with pollen of Gila and of Acala cotton, respectively, in 1919 and with pollen of Acala in 1920.*

Season and character of pollination.	Plants.	F_1 hybrids.	
		Number.	Per cent.
Season of 1919:			
Gila (Egyptian)	206	74	35.9±2.3
Acala (upland)	287	96	33.4±1.9
Difference			2.5±3.0
Season of 1920:			
Acala (upland)	479	143	29.8±1.4

The difference in the percentage of hybrids produced in 1919 by the two foreign pollens was not significant, and it is clear that pollen of another variety of the same species (Egyptian cotton) was not better able than pollen of a different species (upland cotton) to compete with the self pollen. The percentage of hybrids in both cases is very high in comparison with that of the 1916 experiment with Gila pollen.

In a similar experiment in 1920 a number of Pima flower buds were bagged but were not emasculated, so that automatic self-pollination was not interfered with. Early in the afternoon of the day of anthesis abundant pollen of the Acala variety of upland cotton was applied to the stigmas. The resulting seed was planted in 1921. No thinning was done, all seeds which germinated having been allowed to develop. The resulting percentage of F_1 hybrids, as also stated in Table 26, did not differ significantly from that yielded by seed resulting from the corresponding self + cross-pollination in the experiment of 1919.

The percentage of cross-fertilization in all three of these experiments was considerable, notwithstanding that both in time of arrival upon the stigmas and in nearness to the ovary the automatically deposited self pollen would seem to have had a marked advantage. The results therefore tend to confirm the evidence given on preceding pages that conditions at the base of the stigmas are relatively unfavorable for the germination of the pollen or penetration of the tubes.

DEPOSITION OF THE TWO POLLENS SIMULTANEOUS.

An experiment performed in 1919 was designed to determine what percentages of the ovules are fertilized by pollen of the same and of another variety when both sorts of pollen are applied as nearly as possible simultaneously and in as nearly as possible equal quantity. For this purpose a number of Pima cotton flowers were emasculated and bagged in the evening. During the following morning the stigmas were smeared with pollen of the same variety and with pollen of Acala (upland) cotton. Half of the flowers on each date received the upland pollen first, and the other half received the Pima pollen first, but the interval of time between the applications of the two lots of pollen was negligible. This method of applying the two pollens was adopted because of the impracticability of mixing them in approximately equal quantity. The comparative viability of the pollens used in this experiment was not determined, but pollen of Pima and of Acala from plants growing in the same field had been tested in a sugar solution three weeks previously and had shown no appreciable difference in viability.

The seeds obtained from each pollination were planted in 1920, four seeds to the hill, and the rows were not thinned. The number of first-generation Egyptian × upland hybrids in each lot was determined early in July, with the results given in Table 27. Comparison of the percentages of hybrids in hills containing, respectively, one, two, three, and four plants, showed no significant differences. This would indicate that the heterozygotes, in spite of the larger size which they soon attained, had had no special advantage during the germinating and seedling stages (see p. 5).

TABLE 27.—*Hybrids from seeds produced by emasculated flowers of Pima cotton when pollinated first with upland cotton and then with Pima pollen and vice versa.*

Pollination.	Plants.	F_1 hybrids.	
		Number.	Per cent.
Pima × upland + Pima	134	33	24.6±2.5
Pima × Pima + upland	160	21	13.1±1.8
As one array	294	54	18.4±1.5

It will be noted that the percentage of hybrids produced was almost twice as great when the upland pollen was applied first as when the Pima pollen was applied first, and the difference amounted to about three and a half times its probable error, whereas in the double-pollination experiment performed by McLachlan (Table 25) the percentage of hybrids from flowers borne by Egyptian plants was somewhat higher when Egyptian pollen was applied first than when upland pollen was applied first. Possibly in the present experiment the stigmas were so well covered at the first application that many of the pollen grains of the second application were not in contact with the stigmatic surface. It would seem to be a fair assumption that the percentage of hybrids obtained by taking as one array plants resulting from the two treatments represents what would have been obtained if a mixture of both pollens in equal quantity had been applied.

The results of the McLachlan experiment showed that 89 per cent of the ovules of Yuma (Egyptian) cotton had been fertilized by pollen of the same variety when upland pollen was also present on the stigmas. In the present experiment 82 per cent of the ovules of Pima (Egyptian) cotton were fertilized by pollen of the same variety, although both pollens were applied as nearly as possible simultaneously and in equal quantity. The fact that even when upland pollen was applied first only about 25 per cent of the resulting plants were hybrids makes it difficult to avoid the conclusion that on the stigmas of Egyptian cotton pollen of the same variety is strongly prepotent over upland pollen.

It is interesting to compare the percentages of hybrids obtained in the double-pollination experiment (Table 27) with the percentages, as given in Table 26 (1919 experiment), from flowers which had not been emasculated and had had the stigmas pollinated (1) with pollen of another variety of Egyptian cotton and (2) with upland pollen. Notwithstanding the fact that in the latter case automatic self-pollination of the basal or interstamen region of the stigmas was not interfered with, the percentage of hybrids which resulted from either cross-pollination was about twice as great as the average of the percentages from the two double cross-pollinations of emasculated flowers. This further corroborates the conclusion that pollen deposited at the base of the stigmas, as was the self pollen in the unemasculated flowers, is under less favorable conditions than pollen deposited nearer the apex, as was the foreign pollen in the same flowers. In the double pollination of emasculated flowers the pollen of both kinds was deposited over the same stigmatic area.

CONCLUSIONS REGARDING SELECTIVE FERTILIZATION.

The occurrence of selective fertilization in favor of the Pima pollen when this and upland pollen are present in approximately equal quantity on the Pima stigmas is interesting in view of the evidence given on preceding pages that the upland pollen, when applied separately, germinates at least equally well and penetrates the ovary with a rapidity equal to that of the Pima pollen. To account for selective fertilization it seems necessary, therefore, to assume that there is a partial inhibition of the germination or subsequent development of the foreign pollen when pollen of the same variety is also present on the same stigmatic area.

Darwin (*13*, pp. 391, 392), although concluding that pollen from another plant of the same variety or from another variety of the same species "is often or generally prepotent over that from the same flower," obtained evidence that the reverse is true in the case of different (but presumably compatible) species.

> If pollen from a distinct species be placed on the stigma of a castrated flower, and then after several hours pollen from the same species be placed on the stigma, the effects of the former are wholly obliterated, excepting in some rare cases.

The results here described are not in accordance with Darwin's findings, for in the experiments in which foreign pollen was applied to unemasculated Pima flowers, pollen "from another variety of the same species" (Gila) was not prepotent over the self pollen, and pollen of a distinct species (Acala) fertilized a considerable percentage of the ovules although applied several hours after self-pollination had begun. Acala pollen was also able to effect fertilization when applied simultaneously with self pollen to Pima flowers which had been emasculated.

Interesting results as to pollen competition in maize have been reported recently by Jones (*24*, *25*, *26*), who found that in the great majority of the combinations tested more of the ovules were fertilized by self pollen than by foreign pollen when the two kinds were in direct competition and that the "handicap placed upon the foreign pollen is proportional to the germinal unlikeness" (*25*, p. 283), notwithstanding the fact that the weight of the seeds resulting from cross-fertilization and the vigor of the plants grown from such seeds increased with the wideness of the cross. "In proportion as the cross-fertilization benefits the immediate progeny in its development the less effective is that pollen in accomplishing the union" (*25*, p. 271).

The results of the experiments with cotton described in this bulletin agree only partially with those obtained by Jones with maize. It is true that on the stigmas of emasculated flowers of Egyptian cotton, pollen of the same variety was found to be prepotent over pollen of a very distinct type (upland cotton) when the two kinds were in direct competition. On the other hand, when applied to the stigmas of unemasculated Pima flowers, pollen of another variety of the same type (Gila, Egyptian) was not more successful than pollen of another type (upland) in competing with the self pollen. Yet heterosis is far more pronounced in the cross Pima × upland than in the cross Pima × Gila. Furthermore, McLachlan's results (Table 25) indicate that on the stigmas of upland cottons

pollen of a very different type (Egyptian) competes on nearly equal terms with pollen of the same variety.[27]

RELATIVE COMPLETENESS OF INSECT POLLINATION AT DIFFERENT LOCALITIES.

Observation in Arizona has shown that the number of efficient pollinating insects differs greatly in different localities.[28] Bees and other active pollinators are normally abundant among the cotton flowers at Sacaton throughout the summer, and the entire surface of the stigmas is almost invariably well covered with pollen soon after the corolla has opened. On the other hand, observations in the Salt River Valley, at distances of 25 to 40 miles from Sacaton, have shown that insect pollination of cotton there is often much less rapid and complete. The probable explanation is that in recent years an extensive and almost continuous acreage has been planted to cotton, and the insect population is not large enough to insure thorough pollination of all the flowers.

Thus, on July 18, 1919, in a field situated near Tempe in the heart of the cotton-growing district, no pollen grains were observed upon the extrastaminal portion of the stigmas at 9 a. m. and very few at 10 a. m. Late in the afternoon of July 20, 1920, inspection of the same field showed the extrastaminal portion of the stigmas to be free from pollen in most of the flowers, while the remainder bore only a few insect-transported grains. None of the flowers examined showed thorough pollination of the whole stigmatic surface. Two other centrally located fields, one at Phoenix and one near Tempe, which were examined at 5 p. m. on August 5 and at 4 p. m. on August 6, showed similarly deficient pollination. On the other hand, in fields situated on the outskirts of the valley, at Litchfield and at Goodyear, which were examined at noon on the same days, bees and other pollinators were abundant, and the stigmas of the cotton flowers were found to be well covered with pollen.

Experiments were made in 1920 with the object of comparing the relative degree of fertilization by natural pollination in fields where observation had shown, on the one hand, thorough pollination of the entire stigmatic surface and, on the other hand, a deficiency of pollen on the upper portion of the stigmas. It was sought also to ascertain whether in the latter case artificial pollination would increase the degree of fertilization, as compared with that of naturally pollinated flowers.

At weekly intervals during the month of August the stigmas of approximately 100 flowers were smeared with pollen from other Pima plants, and an approximately equal number of flowers were tagged and left to natural pollination. The only difference between the two treatments was the thorough cross-pollination of the entire stigmatic surface of the artificially pollinated flowers, neither lot

[27] Attempts made at Sacaton, Ariz., to determine whether selective fertilization occurs in upland cotton have been unsuccessful, owing to the loss by shedding of nearly all of the bolls from the treated flowers.

[28] A pronounced difference in the abundance of pollinating insects at different localities in Arizona was noted by Cook, McLachlan, and Meade (*12*, p. 34): "At the time of our visits to the fields at Yuma and Sacaton there was a notable difference in the activity of the insects at the two places. Several species of large wild bees that were industriously visiting the flowers at Yuma in September were not seen at all at Sacaton."

having been bagged. These experiments were performed at Sacaton, Ariz., and in a field near Phoenix where observation had shown that even late in the afternoon the upper portion of the stigmas remained relatively free from pollen.

The percentages of bolls matured from each lot of flowers and the mean numbers of seeds per boll are stated in Table 28. The data are given for each day separately and for all four dates as one array.

TABLE 28.—*Results of natural and artificial pollination of unbagged flowers of Pima cotton at Phoenix and at Sacaton, Ariz., in 1920.*

Locality and date.	Naturally pollinated flowers.					Artificially pollinated flowers.				
	Flowers treated.	Bolls matured.	Percentage of bolls matured.	Mean number of seeds per—		Flowers treated.	Bolls matured.	Percentage of bolls matured.	Mean number of seeds per—	
				Boll matured.	100 flowers treated.[1]				Boll matured.	100 flowers treated.[1]
Sacaton, Ariz.:										
Aug. 6....	95	88	92.7±1.8	15.1±0.21	1,400±33	94	82	87.2±2.3	15.1±0.24	1,316±40
Aug. 13...	96	91	94.8±1.5	14.6± .19	1,385±28	91	88	96.7±1.3	14.6± .24	1,411±30
Aug. 20...	91	88	96.7±1.3	16.1± .21	1,556±29	89	87	97.8±1.1	16.3± .18	1,595±25
Aug. 26...	95	93	97.9±1.0	17.7± .15	1,734±23	87	83	95.4±1.5	17.7± .15	1,689±30
All dates..	377	360	95.6± .7	15.9± .11	1,520±15	361	340	94.2± .8	15.9± .11	1,496±16
Phoenix, Ariz.:										
Aug. 6....	91	80	87.9±2.3	11.1± .34	975±39	98	93	94.9±1.5	15.8± .23	1,500±32
Aug. 13...	92	66	71.8±3.2	12.3± .44	883±50	89	85	95.5±1.5	17.3± .23	1,651±34
Aug. 20...	72	62	86.1±2.8	15.3± .29	1,317±50	95	72	75.8±3.0	16.7± .22	1,266±53
Aug. 26...	89	76	85.4±2.5	17.6± .23	1,501±48	94	90	95.8±1.4	17.9± .17	1,716±30
All dates..	344	284	82.6±1.4	14.0± .20	1,157±26	376	340	90.3±1.0	16.9± .10	1,526±19

[1] The probable error of this value was computed by the formula $\sqrt{(Ab)^2+(Ba)^2}$, A±a being the percentage of bolls matured and B±b being the mean number of seeds per boll.

Considering the combined results for the four dates, it appears that the fertilization of the naturally pollinated flowers at Phoenix was significantly inferior to that at Sacaton, the difference in the percentage of bolls matured having been 13±1.6 and the difference in the mean number of seeds per boll having been 1.9±0.23. At Sacaton it is evident that artificial pollination did not result in more nearly complete fertilization than was attained by natural pollination, neither the percentage of bolls matured nor the mean number of seeds per boll having differed significantly in the two lots of flowers. Artificial pollination at Phoenix, on the contrary, significantly increased the degree of fertilization, the increases over the results from naturally pollinated flowers having been for the entire period 7.7±1.7 in the percentage of bolls matured and 2.9±22 in the mean number of seeds per boll. In the mean number of seeds per 100 flowers, a value which integrates the percentage of bolls matured and the mean number of seeds per boll, the increase due to artificial pollination amounted to 32 per cent, indicating that a substantially greater crop both of seed and of fiber[29] might be expected if bees were abundant in the Salt River Valley cotton fields during the blossoming period.

[29] Evidence that the weight of fiber per boll is correlated with the number of seeds has been presented elsewhere (28).

SEASONAL VARIATIONS IN THE RELATIVE COMPLETENESS OF FERTILIZATION.

Comparison of the fertilization attained in different seasons can be made most effectively on the basis of the mean number of seeds per boll expressed as a percentage of the mean number of ovules. Counts made in 1921 on 250 3-celled and on 25 4-celled ovaries of Pima cotton showed the mean numbers of ovules to be 20.6 ± 0.09 and 25.3 ± 0.34, respectively. Since at Sacaton approximately 5 per cent of the Pima ovaries are 4-celled and practically all the others are 3-celled, the mean number of ovules in a random sample of ovaries should be 20.8. The frequency distributions for the number of ovules in the 3-celled and the 4-celled ovaries are given in Table 29.

TABLE 29.—*Frequency distributions of the number of ovules in 3-celled and in 4-celled ovaries of Pima cotton.*

Ovaries.	Number of ovules.															
	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
3-celled	5	8	11	16	24	41	52	44	35	14	....	....	....	....	....	....
4-celled	....	....	....	....	....	....	1	3	3	4	3	2	4	1	3	1

Bolls from various lots of naturally pollinated flowers at Sacaton have been found to contain mean numbers of developed seeds as given in Table 30, which also states the mean numbers of seeds as percentages of the mean number of ovules when taken as 21.

TABLE 30.—*Mean numbers of seeds per boll from different lots of naturally pollinated flowers of Pima cotton at Sacaton, Ariz.*

[The mean numbers of seeds are stated also as percentages of the mean number of ovules.]

Year.	Plants.	Bolls.	Mean number of seeds.	
			Actual.	As percentage of the mean number of ovules.
1915	53	530	16.0 ± 0.12	76
1916	81	810	$16.9 \pm .08$	80
1917	82	820	$17.7 \pm .07$	84
1918	85	850	$16.5 \pm .07$	78
1919	200	200	$16.5 \pm .13$	78
1920	.....	95	$14.2 \pm .21$	67
1920	.....	91	$15.0 \pm .20$	71
1920	.....	360	$15.9 \pm .11$	75
1921	100	[1] 100	$18.6 \pm .13$	89
1921	30	560	$17.3 \pm .09$	82

[1] 3-locked bolls only.

The mean percentage of ovules fertilized, as stated in Table 30, varied from 67 to 89. The fact that both lots of flowers in 1921 gave a significantly higher mean number of seeds than any of the three lots in 1920 indicates that there is considerable variation from year to year in the conditions for fertilization. Reference to Table 28 shows that the conditions vary also during the same season. Con-

sidering only the naturally pollinated flowers, the mean numbers of seeds per boll and per 100 flowers were significantly greater during the second half of August, 1920, than during the first half. Whether the difference, which was much more pronounced at Phoenix than at Sacaton, was caused by more favorable weather or by an increase in the number of pollinating insects is uncertain.

The bolls upon which were computed the mean numbers of seeds given in Table 30, with the single exception noted, were taken at random, without reference to the number of locks. It has been determined by a number of counts which have given practically the same result, that at Sacaton the proportion of 4-locked bolls in Pima cotton slightly exceeds 5 per cent,[30] practically all the others being

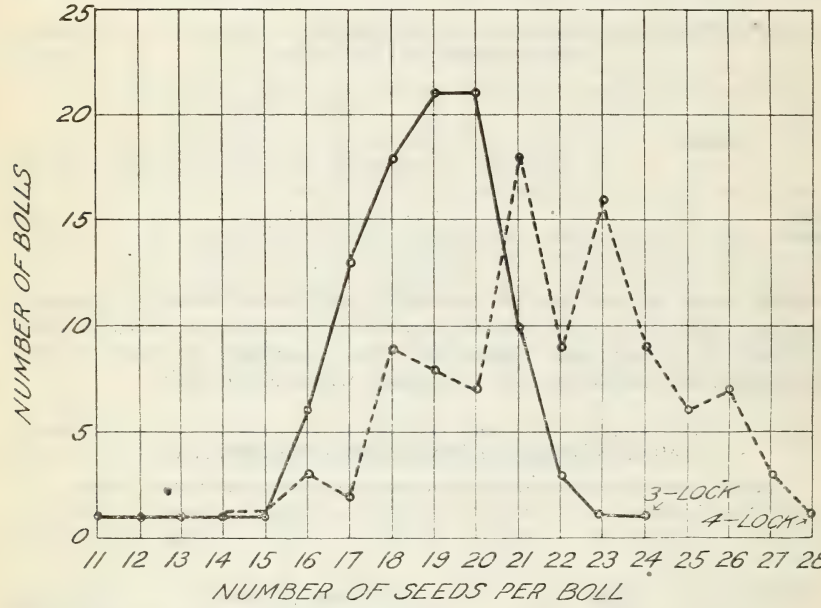

FIG. 4.—Frequency distributions of the number of seeds in one hundred 3-locked and 4-locked bolls on well-grown plants of Pima cotton at Sacaton, Ariz., in 1921. The bolls were collected in pairs, a 3-locked and a 4-locked boll from each plant. The mean numbers of seeds were 18.6 ± 0.13 for the 3-locked bolls and 21.7 ± 0.19 for the 4-locked bolls. The frequency distribution for the 3-locked bolls, shown by the solid line, is much more regular than that for the 4-locked bolls, shown by the dotted line.

3-locked, the number of bolls having two and five locks being negligible. It was found at Sacaton in 1921 by counts on 100 bolls of each lock number taken from as many plants that the average number of seeds per boll was 18.6 ± 0.13 for the 3-locked bolls, and 21.7 ± 0.19 for the 4-locked bolls.[31] The frequency distributions for the number of seeds in the 3-locked and in the 4-locked bolls are shown in Figure 4.

[30] Much higher percentages have been recorded at other localities. Counts made by C. G. Marshall and W. B. Camp on 40 plants in a field of Pima cotton near Bakersfield, Calif., in 1917, showed that in a total of 2,486 bolls 21.3 per cent were 4-locked. O. F. Cook in 1920 counted bolls on 5 plants taken at random in a field near Porterville, Calif., and found that in a total of 62 bolls, 20 (or 32.3 per cent) were 4-locked.

[31] A greater difference in sea-island cotton in the mean numbers of seeds in 3-locked and 4-locked bolls is indicated by data given by Harland (21, Table II, p. 152), which show that the means for 3-locked and 4-locked bolls were 17.4 and 23.2, respectively.

THE INFERIOR FERTILIZATION OF BAGGED FLOWERS.

Bolls from flowers which have been inclosed in paper bags in order to prevent cross-pollination and which have not been pollinated by hand nearly always contain fewer seeds than bolls from open-pollinated flowers. For example, in 1915, the mean number of seeds per boll in 678 bolls from bagged flowers was found to be only 13.6 ± 0.34, as compared with a mean of 16 ± 0.12 in 530 bolls from open-pollinated flowers. In Table 31 are assembled the data from a number of experiments which afforded a close comparison of the relative fertilization of bagged and of open-pollinated flowers, both lots of flowers having opened during the same period and either on the same or on neighboring plants.

TABLE 31.—*Relative completeness of fertilization in bagged and in open-pollinated flowers of Pima cotton.*

Year and treatment of flower.	Number of bolls.	Mean number of seeds per boll.	Year and treatment of flower.	Number of bolls.	Mean number of seeds per boll.
Season of 1916:			Season of 1920:		
Bagged	743	10.9±0.14	Bagged	80	10.7±0.36
Open pollinated	707	15.3± .12	Open pollinated	95	14.2± .21
Difference		4.4± .18	Difference		3.5± .42
Season of 1919:			Bagged	634	11.5± .13
Bagged	168	13.9± .23	Open pollinated	360	15.9± .11
Open pollinated	174	16.6± .25	Difference		4.4± .17
Difference		2.7± .34	Season of 1921:		
Bagged	62	16.5± .25	Bagged	129	15.4± .30
Open pollinated	58	18.2± .22	Open pollinated	560	17.3± .09
Difference		1.7± .33	Difference		1.9± .31

The data in Table 31 indicate in every case very significant inferiority of the bagged flowers in relative completeness of fertilization. In seeking an explanation of this difference the following factors are to be considered:

(1) Exclusive self-pollination of the bagged flowers.
(2) Special environment created by inclosure of the flowers in paper bags.
(3) Pollination of only the lower half of the stigmatic surface in the bagged flowers.

Evidence was given on a preceding page that in Pima cotton self-pollination as compared with cross-pollination does not result in an inferior degree of fertilization. That inclosure of the flowers is not an important factor is indicated by the results of an experiment in 1921. On the same Pima plants a number of flowers were bagged before opening, and others were left unbagged. Neither lot was emasculated, and both were pollinated during the morning of the day of anthesis with pollen from other plants of the same variety, the whole stigmatic surface having been thoroughly covered with pollen. Presence or absence of the bags was therefore the only variable. The data given in Table 32 show that the bagged flowers were as well fertilized as those which were not inclosed.

TABLE 32.—*Fertilization of unemasculated Pima flowers of which the upper portion of the stigmas was thoroughly pollinated with pollen of the same variety, some of the flowers having been inclosed in paper bags and others having been uninclosed.*

Treatment.	Flowers treated.	Percentage of bolls matured.	Mean number of seeds per boll.
Bagged	170	88.3±1.7	17.2±0.16
Not bagged	170	89.4±1.6	17.0± .19
Difference	.....	1.1±2.3	.2± .25

Since the absence of cross-pollination and the environment created by inclosure in bags do not seem to be responsible for the inferior fertilization of the bagged flowers, the third variable, locus of pollen deposition, remains to be considered. Table 33 gives the data of several experiments in which the results of pollinating only the lower halves of the stigmas, as in the case when the flower is bagged and left to automatic self-deposition, are compared with the results of pollinating the whole stigmatic surface. In all of these experiments the area of pollen deposition was the only variable, all flowers having been inclosed in bags but not emasculated, and the artificial pollination of the upper portion of the stigmas having been done either with pollen from the anthers of the same flower (experiments of 1916, 1917, and 1920) or with pollen from another flower on the same plant (experiment of 1921).

TABLE 33.—*Relative completeness of fertilization of Pima flowers in which pollen was confined to the lower halves of the stigmas and of flowers which received pollen on the whole stigmatic surface.*

Year and area pollinated.	Flowers treated.	Percentage of bolls matured.	Mean number of seeds per boll.	Year and area pollinated.	Flowers treated.	Percentage of bolls matured.	Mean number of seeds per boll.
Season of 1916:				Season of 1920:			
Lower	1,143	35.5±1.0	10.8±0.21	Lower	95	84.2±2.5	10.7±0.36
Whole	1,100	45.9±1.0	12.5± .18	Whole	97	86.6±2.3	11.2± .32
Difference	.....	10.4±1.4	1.7± .28	Difference	.....	2.4±3.4	.5± .48
Season of 1917:				Season of 1921:			
Lower	78	90.0±2.3	16.7± .40	Lower	137	94.2±1.4	15.4± .30
Whole	86	74.0±3.2	17.5± .39	Whole	176	90.4±1.5	17.5± .17
Difference	.....	16.0±3.9	.8± .56	Difference	.....	3.8±2.0	2.1± .34

The data in Table 33 show that there was in every case a higher mean number of seeds per boll from flowers of which the whole stigmatic surface was pollinated, although the differences were significant only in the experiments of 1916 and 1921. These data alone do not make it clear whether the inferior fertilization when pollen is confined to the lower half of the stigmas is due to the smaller size of the area pollinated or to less favorable conditions for pollen development in the basal region of the stigmas. Reference to Table 16 shows, however, that confining pollen to half of the stigmatic area when this was the apical half, did not result in diminished fertilization as indicated by the mean number of seeds per boll. It may be concluded, therefore, that the inferior fertilization of bagged flow-

ers which receive pollen only on the lower half of the stigmas is due primarily to this region being relatively unfavorable to the germination or development of the pollen.

BOLL SHEDDING IN RELATION TO POLLINATION AND FERTILIZATION.

A general discussion of the phenomenon of boll shedding would be out of place in this bulletin. The physiological aspects of the subject have been treated by Balls (*8*, pp. 65–75), Lloyd (*37* and *38*), Ewing (*17*, pp. 21–37), and King (*31*, pp. 11–21). It may be well, however, to consider briefly such data as have been obtained at Sacaton, Ariz., concerning the relation between the shedding of bolls and fertilization. The observed percentages of boll shedding in Pima cotton at Sacaton, as recorded in Table 34, are in most cases much lower than have been reported by investigators of sea-island and upland cottons at other localities (*20*, p. 195; *17*, p. 21; *40*). In Pima cotton grown at Phoenix, Ariz., 40 miles distant from Sacaton, King (*31*, p. 19) recorded instances of bolls shed in 1919 ranging from 16.7 to 26.5 per cent.

TABLE 34.—*Boll shedding from flowers of Pima cotton naturally pollinated in different years at Sacaton, Ariz.*

Year of experiment.	Flowers recorded.	Flowers which failed to develop bolls (per cent).	Year of experiment.	Flowers recorded.	Flowers which failed to develop bolls (per cent).
1919	200	13.0±1.6	1920	377	4.4±0.7
1919	69	16.0±3.0	1921	999	10.3± .6
1920	98	3.0±1.2	1921	[1] 4,931	25.1± .4
1920	99	8.1±1.8			

[1] Flowers tagged daily during the period from July 11 to September 15.

It is unlikely that deficient pollination is a frequent cause of boll shedding in Pima cotton. Meade (*40*), working with upland varieties, found that "bolls failed to set unless at least 25 grains of pollen were applied to the stigmas; even with this number only one or two seeds matured in each lock." The records for flowers of Pima cotton which have been bagged to prevent cross-pollination show that the quantity of self pollen deposited automatically upon the basal portion of the stigmas is sufficient to insure, as a rule, the retention and maturation of 80 to 90 per cent of the bolls. In flowers naturally open pollinated at Sacaton, additional large quantities of pollen are conveyed to the stigmas by insects. It is probable that only in rare instances is the number of pollen grains which reach the stigmas fewer than 10 times the number of ovules (Pl. VII). Even where insect pollination is deficient, as in the field at Phoenix where the experiment in artificial pollination was performed, the data given in Table 28 show that the additional pollen applied by hand to the stigmas increased the proportion of bolls retained and matured by only about 9 per cent, while increasing the mean number of seeds per boll by 21 per cent.

Evidence also has been obtained that it is not requisite that many of the ovules be fertilized in order that bolls may be retained and

matured. Thus, in 1917, among 71 bolls which matured from a lot of bagged flowers, 3 contained only 2 seeds, 6 contained only 3 seeds, 3 contained only 4 seeds, and 5 contained only 5 seeds. Hence, in 17 bolls (or 24 per cent of the number which reached maturity) fewer than one-fourth of the mean number of ovules had been fertilized. Counts were made of the number of seeds in 633 bolls which matured from bagged flowers in 1920, and of these 2 contained only 1 seed, 7 contained only 2 seeds, and 14 contained only 3 seeds. Few of the bolls which mature from flowers naturally open pollinated show fertilization of less than half of the average number of ovules. Of 447 such bolls in 1920 only 22 (or 4.9 per cent) contained fewer than 10 seeds. On the other hand, of 633 bolls which matured from bagged flowers during approximately the same period 201 (or 31.7 per cent) contained fewer than 10 seeds.

It has been shown on preceding pages that the pollen of Pima cotton is highly viable and perfectly self-compatible. Evidence has been presented, also, that the number of pollen grains normally reaching the stigmas vastly exceeds the number of ovules and that fertilization of only a few of the ovules is necessary in order to insure retention and maturation of the boll. It may be concluded, therefore, that with this variety, under the conditions existing at Sacaton, deficient pollination and fertilization are not important factors in the shedding of bolls.

INBREEDING IN RELATION TO FERTILITY.

The effect upon cotton of continuous and strictly controlled self-fertilization appears to have been little investigated. Leake and Prasad (*36*, pp. 39–45), working in India with cottons of the Asiatic type, obtained indications that partial sterility, as expressed by the percentage of bolls retained, occurred in the first and later inbred generations and that this tendency could be overcome by subsequent cross-pollination. Different types apparently differed in their tendency to sterility. One type showed a marked tendency to imperfect development of the anthers in the second inbred generation which, however, disappeared in the third generation. In one strain self-pollination with pollen from another flower on the same plant resulted in the retention of a much smaller percentage of bolls than did cross-pollination with pollen from a sister plant; but in another strain of the same type no such difference was observed. The numbers of flowers dealt with in these experiments were too small to afford conclusive results.

A different conclusion was reached by Kottur (*34*), who also worked with the Asiatic type of cotton, in India. He found that sterile anthers, containing no pollen, are of common occurrence. In an unselected open-pollinated stock of the Kumpta variety, of which 500 flowers were examined, sterile anthers were found in all but 128, the proportion of empty anthers having been as high as 43 per cent of the total number in some of the flowers. Controlled self-fertilization during six successive generations did not increase this form of sterility. In fact, it is shown by Kottur's data that in the continuously self-fertilized strain the proportion of the flowers having more than 10 sterile anthers was only 13.6 per cent, as compared with 35.9 per cent in the open-pollinated stock. Kottur also observed that the rate of boll shedding was lower and that the

percentage of ovules which failed to develop into seeds was no greater in the continuously self-fertilized than in the continuously open-pollinated population. Kottur states further that continuous self-fertilization in the Asiatic cottons (*Gossypium herbaceum* and *G. neglectum*) and in American upland cotton (*G. hirsutum*) did not induce sterility.

The effects of inbreeding in Pima cotton have been the subject of investigation at Sacaton, Ariz., the following criteria of fertility having been considered:

Viability of the pollen.
Number of ovules.
Rate of flowering.
Boll-shedding percentage.
Size of the boll.
Weight of seed cotton per boll.
Number of seeds per boll.
Weight of seeds.
Viability of the seeds (germination percentage).
Lint index (weight of fiber per 100 seeds).

Comparison with random samples of the continuously open-pollinated (hence, more or less cross-pollinated) stock has been used necessarily as the measure of fertility in the inbred populations, although it is realized that inbreeding may have been accompanied by segregation, plus or minus, in respect to some or all of these values. There was, however, no intentional selection in the development of the inbred families here dealt with.

POLLEN VIABILITY OF AN INBRED POPULATION.

A family resulting from strict self-fertilization during five successive generations was compared in 1919 with a continuously open-pollinated stock in regard to the viability of the pollen as measured by the rate of ejection of the contents of the grains and by the percentage of the total number ejected in a 5 per cent solution of cane sugar (see p. 22). Flowers were collected at 11 a. m., one from each of five plants in each population, and flowers of the inbred and of the open-pollinated stock were alternated in making the tests. The results, as given in Table 35, show no difference in the average viability of the pollen.

TABLE 35.—*Viability of the pollen from plants of a family of Pima cotton inbred during five generations compared with that from plants of a continuously open-pollinated stock of this variety.*

Plant and flower.	Inbred family.			Open-pollinated stock.		
	After immersion until ejection—		Estimated ejection.	After immersion until ejection—		Estimated ejection.
	Began.	Ceased actively.		Began.	Ceased actively.	
	Seconds.	*Seconds.*	*Per cent.*	*Seconds.*	*Seconds.*	*Per cent.*
No. 1	70	170	95	60	170	95
No. 2	65	110	100	60	170	100
No. 3	70	180	100	70	135	95
No. 4	85	155	93	65	120	100
No. 5	55	120	100	65	140	100
Average	69	147	98	64	147	98

It was observed that in three of the five flowers of the inbred population a few of the pollen grains were very small and did not eject their contents during the period of observation, which was about two and one-half minutes. Only one of the five flowers of the open-pollinated stock had an appreciable number of abnormally small grains, although in this case the proportion of such grains was fully as great as in any of the flowers of the inbred family. Further observations were therefore made upon the occurrence of sterile pollen grains in the inbred and in the continuously open-pollinated population. Examination of a number of flowers of the continuously open-pollinated stock showed that in most cases from 2 to 5 per cent of the pollen grains were very small and presumably sterile, 20 per cent of the pollen in one flower having been of this character. Counts made on pollen from five plants of each population, with the results given in Table 36, afforded no evidence of an increase in the proportion of sterile pollen grains having resulted from continuous self-fertilization.

TABLE 36.—*Flowers of a family of Pima cotton inbred during five generations compared with those of a continuously open-pollinated stock of this variety, showing the percentage of abnormally small and presumably sterile pollen grains.*

Plant and Flower.	Inbred family.			Open-pollinated stock.		
	Number of pollen grains.[1]		Per centage of sterile grains.	Number of pollen grains.[1]		Percentage of sterile grains.
	Total.	Sterile.		Total.	Sterile.	
No. 1	?	0	0	26	1	3.8
No. 2	56	1	1.8	?	0	0
No. 3	?	0	0	30	2	6.7
No. 4	14	2	14.3	36	1	2.8
No. 5	?	0	0	25	3	12.0

[1] The total number of grains in the field of the microscope was not determined if no abnormally small grains were present.

It would be expected that if continued inbreeding had impaired the viability of the pollen, fertilization in the inbred strain would be more nearly complete when the flowers are cross-pollinated than when they are self-pollinated. The data given in Table 20 show, however, that in a family which had been inbred during seven successive generations there was not a significant difference in the mean number of seeds per boll from the cross-pollinated and from the self-pollinated flowers and that a somewhat higher percentage of bolls matured from the self-pollinated than from the cross-pollinated flowers.

NUMBER OF OVULES IN AN INBRED POPULATION.

Counts were made in 1919 of the ovules in 20 ovaries of a family which had been strictly inbred by controlled self-fertilization during five generations and in 30 ovaries of a continuously open-pollinated stock, each ovary having been taken from a different plant. The counts were made upon 3-celled ovaries and yielded the mean and extreme numbers given in Table 37. It is obvious that controlled

self-fertilization during five successive generations, as compared with continuous open pollination, had resulted in no reduction of the number of ovules.

TABLE 37.—*Count of the ovules in the 3-celled ovaries of a family of Pima cotton inbred during five generations compared with those of a continuously open-pollinated stock of this variety.*

Stocks compared.	Mean.	Maximum.	Minimum.
Inbred during five generations	21.0±0.33	24	14
Continuously open pollinated	20.8± .27	24	15

RATE OF FLOWERING, PERCENTAGE OF BOLL SHEDDING, SIZE OF BOLLS, AND NUMBER, WEIGHT, AND VIABILITY OF THE SEEDS IN AN INBRED POPULATION.

A family which had resulted from controlled self-fertilization during seven successive generations was compared in 1921 with a random sample of the continuously open-pollinated commercial stock of the Pima variety, the two populations having been grown in adjacent rows. A record was kept for 88 days of the number of flowers opening daily on every plant of both populations, and from these data were compiled the means given in Table 38, which indicate that there was no significant difference in the potential productiveness of the two populations.

TABLE 38.—*Daily mean number of flowers per plant in a Pima family strictly inbred during seven generations compared with that in a random sample of the continuously open-pollinated stock of this variety.*

Population.	Number of plants.	Mean number of flowers per plant, daily
Inbred	78	1.01±0.02
Open pollinated	84	.97± .02
Difference		.04±. 03

The percentages of bolls shed, the mean numbers of seeds per matured boll, the mean weights of seeds, and the germination percentages of the seeds were determined on 15 plants of each population, well-grown individuals which occupied opposite or nearly opposite positions in the two rows having been selected for comparison. Naturally pollinated flowers which had opened on the same days on both sets of plants furnished the material. This procedure eliminated sources of error which might have arisen from soil heterogeneity or from differences in the weather during the period of development of the flowers and bolls. The data, as given in Table 39 (upper part), show no significant differences between the two populations except in the mean weight of seeds, in which case the difference was in favor of the inbred population.

TABLE 39.—*Pima cotton strictly inbred during seven successive generations compared with a random sample of the continuously open-pollinated commercial stock of this variety.*

Population.	Flowers tagged.	Percentage of bolls shed.	Mean number of seeds per matured boll.	Mean weight of 100 seeds (grams).	Percentage of germination of the seeds.[1]
Inbred	296	11.8±1.3	17.2±0.12	13.6±0.04	90.8±0.8
Open pollinated	367	8.4±1.0	17.1± .12	13.4± .03	90.2± .9
Difference	.....	3.4±1.6	.1± .17	.2± .05	.6±1.2

Population.	Boll dimensions.			Boll weight and lint index.		
	Number of bolls.	Length (millimeters).	Diameter (millimeters).	Number. of bolls.	Seed cotton (grams).	Lint index.
Inbred	25	46.6±0.56	26.8±0.19	105	3.22±0.21	4.90±0.27
Open pollinated	25	45.7± .80	26.1± .19	115	3.04± .06	5.12± .03
Difference	.....	.9± .97	.7± .27	.....	.18± .22	.22± .27

[1] Determined for 500 seeds of each population.

Further comparison of the same populations was made in regard to the length and the greatest diameter of the bolls, the weight of seed cotton in the ripe boll, and the lint index. The determinations were made on bolls from naturally pollinated flowers on five plants in each population, individuals which occupied opposite positions in the two rows having been used and the bolls having been from flowers which had opened during the same period in both populations. Five bolls, judged to be full grown, although not yet open, were measured on each plant. The weight of seed cotton per boll and the lint index were determined separately on from three to five lots of five bolls each from each plant. The units from which were computed the means and probable errors, as given in Table 39 (lower part), were in all cases the averages for the individual plants. The results of this comparison show no significant differences between the two populations.

CONCLUSIONS IN REGARD TO THE EFFECTS OF INBREEDING.

No evidence was obtained that the fertility of Pima cotton had been impaired by strict inbreeding during five or seven successive generations. The inbred families were not inferior to the continuously open-pollinated stocks in viability of the pollen; number of ovules; daily flower production; percentage of bolls retained; size, weight, and seed content of the bolls; weight and viability of the seeds; and abundance of the fiber.[32]

The absence of superior fertility in the continuously open-pollinated populations is hardly surprising in view of the evidence

[32] This comparison of the naturally pollinated commercial stock of Pima cotton, a relatively uniform variety, with the closely inbred (self-fertilized) strain may be regarded as a comparison of restricted or "narrow" breeding with "line" breeding, to use Cook's evolutionary terminology (*10*, p. 9). The evidence here presented that the closest inbreeding during seven generations resulted in no diminution of fertility does not prove that such effect might not be shown eventually. From Cook's point of view, "though all forms of restricted descent lead ultimately to degeneration, the decline may be exceedingly slow and gradual if methods of line breeding are followed" (*10*, p. 38).

given on preceding pages that in cotton most of the ovules normally are self-fertilized. This would be expected to result in a degree of "immunity" to the supposedly injurious effects of continued inbreeding, comparable in some degree to that of wheat and other plants in which cross-fertilization rarely takes place under natural conditions.

The hypothesis of a direct physiological effect of inbreeding is rejected, however, by recent investigators (*9*, *15*, *23*, *45*), who attribute the often injurious results to segregation of deleterious recessive factors or to elimination of favorable dominant factors upon which depends the vigor of heterosis. To quote Jones (*23*, p. 95), "whether good or bad results from inbreeding depends solely on the constitution of the organisms before inbreeding is commenced." As stated by East and Jones (*15*, pp. 139, 140): "The only injury proceeding from inbreeding comes from the inheritance received. The constitution of the individuals resulting from a process of inbreeding depends upon the chance allotment of characters preexisting in the stock before inbreeding was commenced. If undesirable characters are shown after inbreeding, it is only because they already existed in the stock and were able to persist for generations under the protection of more favorable characters which dominated them and kept them from sight." Substantially the same idea is expressed by Stout (*45*, p. 124) as follows: "The accumulation of evidence that inbreeding is not necessarily injurious has led to the view that decreased vegetative and reproductive vigor in inbred stock is due to an inherently weak constitution existing before inbreeding was begun."

The assumption seems justifiable that in Pima cotton factors contributing to low fertility had been eliminated in large part in the ancestry of the plant which gave rise to the variety or by the subsequent selection and isolation which resulted in development of the present highly uniform commercial stock. The fertility of the latter may be regarded, therefore, as due to segregation rather than to heterosis.

SUMMARY.

The three principal types of cotton grown in the United States, upland, sea island, and Egyptian, although very different in their botanical characters, intercross readily, giving rise to hybrids which are extremely fertile in the first generation.

When any two of these types or any two varieties of the same type of which the hybrid offspring is easily distinguishable are grown in close proximity in a locality where pollinating insects are abundant, the proportion of vicinists (natural hybrids) in populations grown from the resulting seed seldom exceeds 20 per cent and is often much lower. Upland cotton usually produces more vicinists than Egyptian cotton when each type is equally exposed to cross-pollination by the other.

From the point of view of maintaining varietal purity, a very small proportion of vicinism must be regarded as a menace. A few accidental hybrids, unless they are promptly eliminated, will eventually contaminate the stock.

The percentage of recognizable vicinists does not indicate the total extent of cross-fertilization occurring under natural conditions, many

of the ovules being fertilized by pollen from other plants of the same variety, but even when single plants of Egyptian or of upland cotton were scattered through a field of the other type and the conditions were so controlled as to make it highly probably that all seeds not derived from self-fertilization had resulted from cross-fertilization by pollen of the other type, the maximum number of vicinists in populations grown from these plants did not exceed 35 per cent. The average percentage of cross-fertilized ovules under these conditions was 12 in Pima (Egyptian) and 28 in Acala (upland).

The cotton flower is large and showy, and the reproductive organs are readily accessible to pollen-carrying insects attracted in large numbers by the abundant production of nectar and of pollen. One might therefore expect cross-fertilization to predominate over self-fertilization. The structure of the flower, however, is well adapted not only to cross-pollination by insects but to automatic self-pollination from the uppermost anthers. These are in contact with the base of the stigmas in Egyptian cotton and with practically the whole stigmatic surface in many varieties of upland cotton.

In the Egyptian type only the extrastaminal portion of the stigmas is accessible to foreign pollen, the basal region being effectively screened by the upper anthers. Experimental evidence was obtained that in the Pima variety cross-fertilization does not occur when the extrastaminal portion of the stigmas is excised. In upland cottons, on the other hand, the entire length of the stigmas is accessible to foreign pollen, and excision did not prevent cross-fertilization.

The corolla of Pima cotton commences to open about an hour after sunrise and continues to expand during the next four hours, the maximum aperture usually being attained about 10.30 a. m. In upland cottons an aperture first appears at about the same time as in Pima, but the subsequent expansion is more rapid. In both types the flower is of brief duration, the petals beginning to wilt and the pistils to lose their turgor before sunset on the day of anthesis.

The opening of the anthers and release of pollen in Pima cotton begin somewhat earlier than the expansion of the corolla. In upland cottons, as a rule, corolla and anthers commence to open almost simultaneously, although not infrequently the upland anthers are found to be still closed when the corolla is open sufficiently to admit insects. Even in Pima cotton, however, very little self pollen is deposited automatically upon the stigmas before the petals have begun to unfold.

The viability of the pollen of Pima (Egyptian) and of Durango (upland) cotton, as measured by the rate of ejection of the cell contents when the grains are immersed in a sugar solution, is low during the early hours of the morning, begins to increase rapidly between 8 and 9 a. m., and shows a gradual decline after midday. Inclosure of the flower in a paper bag greatly prolongs the period of viability.

Pollen extracted from the anthers and applied to the stigmas the evening before anthesis showed a very limited ability to effect fertilization. On the other hand, the viability of pollen collected from bagged flowers 26 hours after the beginning of anthesis, as indicated by the ability to effect fertilization, was still considerable, although greatly inferior to that of fresh pollen.

The style of the Pima cotton flower normally is lost by abscission within 36 hours after the beginning of anthesis. In bagged flowers it was found that fertilization could be effected 24 hours after the be-

ginning of anthesis in fewer than 10 per cent of the flowers tested, the indication being that the stigmas do not remain receptive much longer than this even when the flowers are protected by inclosure in bags.

Self pollen being automatically deposited in Pima cotton upon that part of the stigmas which is nearest the ovary and foreign pollen being excluded from this zone by the dense girdle of stamens, the self pollen would seem to have a decided advantage in the distance to be traversed by the tubes in reaching the ovary. Computation from rather unsatisfactory data as to the average rate of growth of the pollen tubes suggests that, other things being equal, pollen tubes from self grains might reach the ovary 3½ hours before penetration could be effected by foreign pollen deposited higher on the stigmas. Evidence has been obtained, however, that the conditions for pollen development are less favorable in the basal than in the apical region of the stigmas, and it is therefore to be doubted that the locus of pollen deposition is an important factor in the preponderance of self-fertilization in Pima cotton. The doubt is increased by the fact that in upland cottons, in which also self-fertilization preponderates, the whole stigmatic surface is accessible to both self pollen and foreign pollen.

The automatic deposition of self pollen upon the stigmas of Pima cotton does not begin long in advance of the first arrival of insect-carried pollen, and in upland cottons the first arrival of pollen from both sources seems as a rule to be virtually simultaneous. It may be concluded, therefore, that the time of arrival of the pollen does not determine the relative frequency of self-fertilization and of cross-fertilization.

By controlling conditions so as to prevent the access of foreign pollen not readily distinguishable from the self pollen, but so as not to interfere with natural cross-pollination, it was demonstrated that a large proportion of the pollen transferred to the stigmas by insects is derived from the anthers of the same flower. As much self pollen is also deposited automatically, preponderance of self-pollination would seem to be an important, if not the principal, factor in the preponderance of self-fertilization.

When the two types are growing side by side, the quantity of Pima pollen deposited upon the stigmas of upland cottons exceeds the quantity of upland pollen deposited upon the Pima stigmas, a fact which helps to explain the greater frequency of upland × Pima than of Pima × upland vicinists. The habits of the pollinating insects and the relative rates of opening of the corolla and anthers in the two kinds of cotton seem to account for this difference in cross-pollination. Many of the Pima flowers are entered by honeybees just as the petals begin to unfold and at a time when little upland pollen is available for transfer, the anthers of the upland flowers being for the most part still closed. In entering the Pima flower in this early stage of expansion the insect comes into contact with the stigmas and stamens, depositing and taking up pollen. Later in the morning, when upland pollen is available in quantity for transfer, the Pima corollas are open to a degree which allows the insects to enter and leave the flower without touching the reproductive organs. Insects coming from Pima flowers which have just begun to open are often loaded with pollen, the Pima anthers usually being well open when the ex-

pansion of the corolla begins. As a result Pima pollen is frequently deposited in upland flowers before the anthers of the latter have opened.

Insect pollination of cotton at Sacaton, Ariz., is effected principally by Hymenoptera, the honeybee and wild bees of the genus Melissodes being the most important species. Certain Coleoptera and Hemiptera also have been observed to carry pollen, but these insects probably are of very minor importance as pollinators.

By applying the different kinds of pollen separately to different flowers it was sought to ascertain whether different degrees of fertilization in Pima cotton result from self-pollination as compared with cross-pollination within the variety and from cross-pollination within the variety as compared with cross-pollination with more foreign pollen. The results were negative so far as concerns cross-pollination within the Egyptian type, there having been no consistent differences in the degree of fertilization attained, whether the stigmas received self pollen, pollen from other plants of the Pima variety, or pollen of another variety of the Egyptian type. On the other hand, somewhat better fertilization of the Pima flowers was obtained in several cases with pollen of a very different type of cotton (upland) than with pollen of the same variety.

An experiment in which Pima flowers were pollinated, some with Pima and others with upland pollen, gave no satisfactory evidence of a difference in the relative growth rate of the pollen tubes of the two types.

Selective fertilization, in favor of the related pollen, has been found to occur when pollen of the same variety is in competition with pollen of another Egyptian variety or with pollen of upland cotton on the stigmas of Egyptian cottons. On the other hand, the results of an experiment performed by Argyle McLachlan indicated the absence of a corresponding prepotency of upland pollen on the upland stigmas, and as most of the ovules normally are self-fertilized in both types of cotton, it remains doubtful in what degree selective fertilization contributes to the greater frequency of self-fertilization.

As both Pima and upland pollen when applied separately to the stigmas of different Pima flowers appear to develop their tubes with equal rapidity and as the upland pollen is at least equally efficient in accomplishing fertilization, it seems necessary, in order to explain the fact of selective fertilization when the two pollens are in direct competition, to assume an inhibiting influence of pollen of the same variety upon the more foreign pollen.

In localities where bees are not numerous in the cotton fields comparatively little pollen reaches the upper part of the stigmas of Pima flowers. Under such conditions artificial pollination resulted in a marked increase in fertilization; whereas at Sacaton, Ariz., where the cotton flowers are much frequented by bees, artificial pollination did not affect the degree of fertilization.

The degree of fertilization resulting from natural pollination has been found to differ in different seasons and at different times in the same season, as well as at different localities.

Flowers inclosed in paper bags in order to exclude foreign pollen and left to automatic self-pollination are nearly always less well fertilized than open-pollinated flowers, but by artificial pollination

of the upper portion of the stigmas of bagged flowers fertilization equal to that of similarly pollinated but uninclosed flowers was attained. This proved that the limitation of pollen deposition to the lower half of the stigmas and not the environment created by the inclosure of the flower is responsible for the relatively poor fertilization of bagged flowers.

Proportions of boll shedding in Pima cotton ranging from 3 to 25 per cent have been recorded at Sacaton for different years and for different lots of plants. It is improbable that deficient pollination and fertilization are primarily responsible for boll shedding at this locality, evidence having been obtained that the pollen of Pima cotton is highly viable and perfectly self-compatible, that the flowers almost invariably receive pollen far in excess of the probable requirement for complete fertilization, and that only a few of the ovules need be fertilized in order that the boll may be retained and matured.

Inbreeding Pima cotton by controlled self-fertilization during five successive generations resulted in no diminution in the viability of the pollen or in the number of ovules. Similar inbreeding during seven generations brought about no reduction in the daily rate of flowering, in the percentage of bolls retained, in the size, weight, and seed content of the bolls, in the weight and viability of the seeds, or in the abundance of the fiber, as compared with those of the continuously open-pollinated stock. It is concluded that deleterious factors had been eliminated in the ancestry of the Pima variety and that its present state of fertility is due to segregation rather than to heterosis.

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